A STUDY OF THE FACTORS GOVERNING THE ISOLATION OF SALMONELLAE FROM INFECTED MATERIALS AND THE APPLICATION OF IMPROVED TECHNIQUES TO EPIDEMIOLOGICAL PROBLEMS

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INTRODUCTION

In a public health laboratory specimens of widely differing nature may be submitted with a request that they be examined for salmonellae. Such specimens may be human or animal faeces, human and animal food stuffs, sewage, polluted water, swabs from the surface of machinery used to mix raw materials compounded into an edible end product, swabs from gulleys draining spilt raw materials in food manufacturing premises and swabs from open gulleys in abattoirs. The numbers of salmonellae in such a diversity of specimens differ greatly as does the associated bacterial flora. A method regarded as highly suitable for the examination of faeces in which the number of salmonellae may be very large (Thomson 1954), may not be equally suited to the isolation of small numbers of these organisms from the highly varied bacterial flora present in sewage, polluted water or infected food. Methods which are effective for isolating salmonellae pathogenic to human beings may not be equally successful when employed for salmonellae which are relatively host specific to animals. Salmonellae whose vitality is diminished by survival outside the animal or human body may differ in their cultural properties from fully viable salmonellae directly passed from the intestine or directly isolated from the internal organs.

My interest in the isolation of salmonellae dates back to an investigation into the sporadic

occurrence of cases of typhoid fever in a Welsh valley (Jones 1949, 1952; Harvey 1957). The technique of typhoid isolation from polluted river water was founded on this investigation and has been further modified in more recent incidents where water polluted with Salmonella typhi has been submitted for examination. The practical techniques have evolved simultaneously with the epidemiological necessity of tracing sources of typhoid fever, and of rendering the streams and rivers free of this dangerous pollution.

The use of sewer swabs to survey Cardiff City for latent sources of enteric fever (Harvey and Phillips 1955), has led to modifications of technique and modifications in the interpretation of the results obtained. The actual method of swabbing sewers has been the subject of investigation and variations in the sampling process have been devised to suit the geographical area under survey. The use of the gauze swab has been extended in its application to drains unconnected with sewers, which collect the raw materials spilt on the floor of food premises (Harvey 1957; Harvey and Phillips 1961). This investigation has given me an insight into the entry of salmonellae into certain food premises. From the above it will be realised that the practical application of improved techniques for salmonella isolation is a very real one.

This thesis is divided into two parts. Part I discusses certain problems of technique which are considered relevant to the isolation of salmonellae from highly contaminated material. Part II illustrates the

use that can be made of an efficient technique in the investigation of the epidemiology of salmonellosis. Much of the work in both Parts I and II has been published, and many of these publications have been joint papers. This has been necessary and welcome as many samples submitted have been taken by members of individual health departments. Without the co-operation of these departments the investigations would have been quite impossible.

The writing of the papers and this thesis have, however, been my own personal responsibility and records work performed or directed by myself.

PART I

CURRENT METHOD OF EXAMINATION OF MATERIALS FOR SALMONELLAE

The usual examination performed involves the use of selenite F broth and/or tetrathionate broth as enrichment media and deoxycholate citrate agar and Wilson and Blair's bismuth sulphite agar as plating media. Times of incubation in enrichment media are often stated as 18 - 24 hours. Times of incubation of plating media are often 24 - 48 hours. The temperature of incubation is usually 37°C. The inoculum of the infected material is not always stated, although it is highly relevant to the results. It is felt that certain factors of considerable importance to the successful examination of material infected with salmonellae are insufficiently studied and elucidated.

These factors may conveniently be set out in the form of questions, as follows :-

I Is the incubation temperature of the enrichment cultures important ?

II What is the effect of changing the size of the inoculum of infected material used for direct plating and for enrichment culture ?

III To what extent is the time of incubation of the enrichment medium relevant to the isolation of salmonellae ?

IV How many subcultures should be made from the enrichment medium ?

V Are deoxycholate citrate agar and Wilson and Blair's bismuth sulphite agar so much superior to a simple brilliant green taurocholate agar that one can

depend exclusively on their use ?

VI Is pre-incubation of infected material in an unselective nutrient fluid medium before selective fluid culture of any value ?

VII Is a change in the concentration of the primary selective agent in the enrichment medium desirable for the isolation of certain salmonellae ?

VIII In specimens yielding many colonies of salmonellae in Phase II, can the technique of Phase II → Phase I change be improved ?

IX When dealing with specimens infected with several salmonella serotypes, has the technique of examination a bearing on the number of serotypes isolated from a single specimen ?

X What enrichment medium is most suitable for routine salmonella isolation ?

These then are the problems studied in salmonella isolation. Often an incomplete answer has been obtained to the above questions. Nevertheless, it is felt that by means of these studies I have developed a reasonably suitable technique for the examination of the diverse samples submitted to the laboratory and for the various surveys conducted in Part II of this thesis.

I IS THE INCUBATION TEMPERATURE OF THE ENRICHMENT CULTURE IMPORTANT ?

I must distinguish between the optimum temperature at which an organism grows in pure culture in an unselective fluid medium and the optimum temperature for its recovery from a mixture of bacteria in a selective fluid medium. The former temperature is, of course, in the region of 37°C. for most pathogens. The latter temperature depends on the selective medium employed, the type of organism it is desired to isolate and the numbers of that organism present in the sample.

My interest in the use of selenite F incubated at temperatures above 37°C. arose in an attempt to discover a suitable enrichment process for the isolation of Shigella sonnei. This organism is stated to grow well at 45°C. (Braun and Weil 1928). This method was a failure as far as Shigella sonnei was concerned, but curiosity prompted me to try the method out with salmonella infected sewage. The results were encouraging. Further tests showed that most salmonellae encountered in a busy routine laboratory would grow at temperatures considerably above 37°C. (Harvey and Thomson 1953). Salmonella typhi is a notable exception to this rule, confirming the findings of others (Browning and Mackie 1937). Although the upper limit of temperature at which some members of the salmonella group will grow is in the region of 47°C. (Haines and Elliot 1944), it does not follow that they are capable of multiplication at such temperatures in a selective fluid medium. Even if high temperature incubation was advantageous with one enrichment medium, it cannot

necessarily be assumed that the method would be equally satisfactory with all enrichment media.

I decided to work with selenite F broth on the grounds that many authors regard it as being the enrichment medium of greatest general utility and range in salmonella isolation (Hobbs and Allison 1945a; Moore 1948, 1950; Williams Smith 1952).

Results

(a) Stools

The occurrence of an extensive epidemic of paratyphoid fever due to Salmonella paratyphi B provided me with sufficient material to test out the method. A suspension of faeces was made in water and an equal volume inoculated into two tubes of selenite F broth. One tube was incubated at 37°C. and the other at 43°C. After 24 hours the cultures were plated onto brilliant green MacConkey's medium (Wilson and Darling 1918). This medium is particularly suitable for comparative trials, as it is very standard in its behaviour. Batch-to-batch variation is rare. The role of other plating media for subculture will be discussed later.

Two hundred positive specimens were examined with the following results :-

TABLE I

Positive at 37°C., positive at 43°C.	147
Positive at 37°C., negative at 43°C.	7
Negative at 37°C., positive at 43°C.	31
Negative at 37°C., negative at 43°C.	15

The results of 15 specimens negative at both temperatures of incubation in selenite F broth are included,

as other methods of examination used routinely in the laboratory had shown them to be positive.

The figures show an advantage in favour of incubation at 43°C., but a greater benefit was the purity of the growth on subculture, as this rendered identification easier. A note was made of this in the 147 specimens positive by both methods. In 81 instances, the subculture from the selenite F broth incubated at 43°C. was purer than that from the selenite F incubated at 37°C. The reverse was true in only eight instances, and in 58 specimens there was no difference.

Large numbers of specimens of faeces were incubated at temperatures between 37°C. and 44°C., but a temperature of 43°C. proved best. A rise in temperature of one more degree to 44°C. resulted in a considerable reduction in the number of positive isolations. If a water-bath cannot be accurately maintained at 43°C., it would be better to set it at 42°C.

Although the higher temperature of incubation enhanced the value of selenite broth as a selective medium, it was unable alone to exert such an influence on ordinary broth so as to render it selective.

From the above experiments it would appear that for Salmonella paratyphi B, at least, incubation of the enrichment cultures at 43°C. had definite advantages. It remained to be seen if the method was suitable for the isolation of other salmonellae.

(b) Sewage

Urban sewage is a prolific source of salmonellae and is a convenient material to evaluate the relative

efficiency of techniques for their isolation. Sewage is usually sampled in this country by the gauze swab devised by Moore (1948). Such swabs are merely folded pieces of gauze tied with string or wire and immersed in the flowing sewage for varying lengths of time. Prolonged exposure to the flowing sewage, e.g., for seven days is regarded as compensating for any intermittency of excretion on the part of a salmonella carrier.

The swabs were received in the laboratory in 1lb. wide-mouthed screw-cap glass containers. The fluid was squeezed out of these swabs on arrival and a measured quantity of fluid was added to the same quantity of double strength selenite F broth. Duplicate cultures were made and one member of each pair was incubated at 37°C., while the other was incubated at 43°C. Subcultures were made only at 24 hours on to brilliant green MacConkey medium (Wilson and Darling 1918). Deoxycholate citrate agar (Leifson 1935; Hynes 1942) and Wilson and Blair's bismuth sulphite agar.

TABLE II

Specimens of Sewage

Summation of positive results on all three plating media subcultured from 37°C. selenite	34
Summation of positive results on all three plating media subcultured from 43°C. selenite	64
Positives missed in selenite incubated at 37°C.	30
Positives missed in selenite incubated at 43°C.	0

The most striking fact was that no positive results were missed by incubating at 43°C., while 30 extra positives were diagnosed. Note that these results refer to enrichment media subcultured once only at 24 hours.

A later series of observations on the isolation of salmonellae from gauze swabs placed in abattoir drains completely confirmed these results. The results are given in Tables V and VI.

The use of 43°C. as an incubation temperature for selenite F broth enrichment would, therefore, appear to be sound for the type of specimen considered in this thesis.

II WHAT IS THE EFFECT OF CHANGING THE SIZE OF THE INOCULUM OF INFECTED MATERIAL USED FOR DIRECT PLATING AND FOR ENRICHMENT CULTURE ?

The size of the inoculum of infected material and the effect of altering it has to be considered in relation both to the plating medium and to the enrichment medium. I shall consider the effect of change of inoculum sown on to the plating medium first and into the enrichment medium subsequently.

(a) Size of inoculum used for plating

This inoculum may either be from the original stool, or as a subculture from the enrichment medium. Faeces from a salmonella excreter does not have a homogenous distribution of the pathogens throughout its bulk. This has been fully demonstrated by the work of Krüger (1951) who compared the results obtained by inoculating four plates directly with solid or semi-solid faeces, with those obtained by inoculating one plate with a small quantity of a homogenous suspension of a large portion of the stool in saline. The one plate sown from the suspension gave better results than the four plates sown with portions of the stool.

There is a limit to the size of infected material that any one plate can accept, as faecal detritus interferes with colonial development (Crone 1948) and colonial morphology may be so interfered with on certain selective media by over crowding, that differentiation of pathogen and non-pathogen may be impossible (Moore 1950). Jones (1936) has also pointed out, that over inoculation of a selective medium with non-pathogenic organisms, normally

suppressed, will allow their growth although they would be completely inhibited if plated in smaller numbers. These difficulties may be circumvented by multiplying the number of plates sown with the infected material and this device has been discussed by Houston (1914) in the isolation of Salmonella typhi from sewage and has been recommended as a practical procedure by Gunther and Tuft (1939). This multiplication of plates, however, considerably increases the work of the laboratory and the tendency in this country would appear to favour the use of dilutions of a suspension of stool for plating inocula rather than the opposite procedure of increasing the quantity of faeces plated (Thomson 1954). My own experience with typhoid carriers has indicated that dilution of a stool may sometimes produce a negative result, while the use of undiluted faeces as inoculum gives a positive isolation. This has been confirmed by Dixon (1961), for Salmonella typhi-murium.

I have been considering above the arguments for and against increasing the inoculum of stool used in direct culture. To some extent the same arguments apply to inocula from enrichment culture. It is not sufficiently realised that the salmonellae present in an enrichment medium may be so scanty, that they may be absent from the normal sized inocula used in subculture. Yet this point was considered as early as 1906 by Loefler in relation to the time of subculture from an unselective fluid medium. I have taken an enrichment culture of a stool on one occasion and have subcultured it after 24 hours incubation at 37°C. on eight separate plates. Only two out of the

eight plates were positive. If I had taken eight inocula from the enrichment broth and plated them on to one single plate it is quite possible that a positive result would have been obtained. On the whole, however, it is probably safer to multiply the number of plates inoculated, than to increase the inocula placed on a single plate. Many laboratories use several different selective plating media for subculture, which comes to the same thing. This phenomenon very probably accounts for the occasional finding of only one of the selective plating media to be positive.

(b) Size of the inoculum of the infected material cultured in the enrichment medium

Enrichment media have one outstanding advantage over selective plating media in that they will accept a larger inoculum of infected material. That there is an advantage to be gained by increasing the inoculum of the infected material, cultured in enrichment media, is gathered from various sources. Thus Hobbs and Allison (1945,a), used heavy inocula of faeces in examining stools for Salmonella typhi, and Haines, Elliot and Tomlinson (Report 1947) more than doubled the number of samples of dried egg from which salmonellae were isolated, by increasing the size of sample from 10 gm. to a whole packet of egg. More recently Galton, Lowery and Hardy (1954), found that in the examination of pork sausages for salmonella, the number of positive specimens increased, with the size of inoculum, up to the point where the selective action of the enrichment medium was interfered with by an excessive amount of meat.

In any comparison of techniques, it cannot be too frequently stressed, that the demonstration of a difference between the methods chosen, lies to a considerable extent in the nature of the material selected for examination. If heavily infected material is chosen, there is little chance of demonstrating that one procedure of culture is better than another. Acute cases of salmonella infection and early convalescent excreters, who are harbouring large numbers of salmonellae, are, therefore, entirely unsuitable for such studies. In late convalescence, however, when the pathogens are scanty and are irregularly distributed throughout the stool mass, the faecal material is ideal for evaluating optimal isolation procedures. Such stools were available at the end of the Paratyphoid B epidemic which occurred in South Wales in 1952 (Thomson 1953).

Two series of stool specimens were examined. In the first series, inoculation of selenite F broth with a large loopful of faeces (5mm. diameter loop) was contrasted with inoculation of 1c.c. of a dense suspension of faeces into selenite F broth. Identical specimens were used. Inoculation of solid faeces yielded 99 positives. Inoculation of 1c.c. of faecal suspension yielded 154 positives. The selenite F broths were only once sub-cultured at 24 hours. The second series of 51 positive stools was examined at the very end of bacteriological clearance. In this series the entire specimen was suspended in selenite F broth after a routine inoculum of solid faeces into selenite F had been made. The specimens were, therefore, paired as before. The suspension of

stool in enrichment broth was of the nature of a thin paste. The paired samples were incubated at 37°C. for 24 hours and were then subcultured on brilliant green MacConkey medium. The results were as follows :-

TABLE III

51 Positive Stools

Number of positive specimens from selenite F broth inoculated with solid faeces (A)	33
Number of positive specimens from selenite F broth inoculated with entire remaining stools sample (B)	46
A + B -	5
A - B +	18

These two series demonstrate the advantage of increasing the inoculum into the enrichment medium. The experience of Pilsworth (1960) with sewage contamined with Salmonella typhi, and of Dixon (1961) with stools containing Salmonella typhi-murium is in agreement with my own preference for undiluted inocula in selenite F broth.

The occurrence of 5/51 samples which were negative by the heavy inoculation technique is interesting and raises a most important question in salmonella isolation. Guth (1916), remarked on the fact that small inocula into selenite broth might produce positive results when heavy inoculation failed to do so. I think that the explanation lies in Leifson's paper on selenite fluid media (1936). Leifson pointed out that selenite F broth seeded with a heavy inoculum of faeces was better subcultured at 36 hours than earlier. A lesser inoculum might often produce a positive result after 18 - 24 hours subculture. This clear exposition of the relation between size of inoculum and

optimum subculture time has received too little attention. It is true that Leifson was discussing the isolation of Salmonella typhi, yet I believe that the same explanation holds good for other salmonella serotypes.

A limited number of experiments on sewage, sewage polluted natural waters and Indian crushed bone, have again demonstrated the advantages of heavy inocula in enrichment media. It is necessary, however, to compensate for the possible late growth of salmonellae, due to heavy inoculation of the enrichment medium, by subculture at 24, 48 and 72 hours.

III TO WHAT EXTENT IS THE TIME OF INCUBATION OF THE ENRICHMENT MEDIUM RELEVANT TO THE ISOLATION OF SALMONELLAE ?

Loefler in 1906 calculated, that for the isolation of Salmonella typhi from 100c.c. of fluid culture medium initially sown with 1 - 2 organisms, the following numbers of plates would be necessary at various times :-

After	10 hours	25 plates	)	Using 1/10 of a loopful as subculture inoculum to each plate
"	12 "	10 "	)	
"	14 "	3 "	)	
"	16 "	1 "	)	

The conclusion ultimately arrived at, was that not less than 18 hours culture at 37°C. of material containing scanty salmonellae was essential to ensure that an inoculum of suitable size for plating (1/10 of a loopful) contained Salmonella typhi. In this work Loefler advocated the use of malachite green enrichment media. He may be regarded as having initiated the serious consideration of an enrichment process. In 1913, Torrey, and Browning, Gilmour and Mackie independently described fluid enrichment media containing brilliant green. Torrey advocated subculture at 24 hours, while Browning, Gilmour and Mackie suggested the use of both 24 hours and 48 hours for subculture times. In a later paper, Browning, Mackie and Smith (1914 - 15), again advocated the subculture of their tellurite brilliant green enrichment medium at 48 hours, if negative by plating at 24 hours. Krumwiede, Pratt and McWilliams (1916), as a contrast tentatively discussed the advantages of short time exposure (1 - 3 hours) of typhoid-containing faeces to brilliant green broth, before subculture. They were apparently disappointed with their results and ended by recommending

that the enrichment cultures be incubated for 18 hours. Topley and Fielden (1922), investigated the growth of a mixed bacterial flora in nutrient broth and demonstrated that the dominant species present in the culture varied in a more or less orderly manner, one species being replaced by another, and this in its turn giving place to a third. These authors pointed out that prolonged subculture might have certain advantages when it was desired to isolate one organism from a mixture. The subculture times were at 24 hours incubation, and thereafter twice weekly to the 60th day, followed by weekly subculture in certain cases. This principle of the orderly replacement of one bacterial type by another is just as valid for specimens containing several salmonella serotypes, or mixtures of salmonellae and arizona paracolons, as will be seen in a later section. (Harvey and Price 1962). Waldhecker (1935), studied the isolation of Salmonella typhi from a tetrathionate brilliant green bile fluid medium and stated that positive isolations were increased by subculture at 5 and 10 hours instead of at 20 hours only. Knox, Gell and Pollock (1943) on the other hand, pointed out that it would be possible to explain the varying results obtained by plating out from tetrathionate broth at different intervals of time, by the progressive diminution in the selectivity of the medium due to tetrathionate reduction. Reduction of tetrathionate results in the formation of thiosulphate which inhibits the growth of Salmonella typhi, but it encourages the multiplication of E.coli and Proteus vulgaris which will govergrow the pathogen. Martin (1947) recorded the fact that occasionally

72 to 96 hours incubation at 37°C. was necessary to isolate Salmonella paratyphi B from polluted river water cultured in a modified Müller's (1923) tetrathionate broth. Williams Smith (1952), in his work on culture media for faecal salmonellae, suggested that the optimum time of subculture from tetrathionate broth (Rolfe 1946 modified) was between 24 and 30 hours. Thereafter the efficiency of this medium decreased. The optimum time of subculture from selenite F on the other hand was at 30 hours, but in contrast to tetrathionate medium there was no falling off of efficiency at 36 and 48 hours. He came to the conclusion that while multiple subculture was necessary when tetrathionate was used, it was not so essential with selenite F, since after the optimum incubation time was reached the number of positive specimens did not decrease. Earlier subculture than 15 hours from either tetrathionate or selenite was found to be valueless. It is interesting that Robinson (1958), tended to regard earlier subculture than 18 hours to be of considerable value in the isolation of Salmonella typhi and subculture between 18 - 24 hours as the optimum subculture time for the isolation of Salmonella paratyphi B.

Recently the use of prolonged subculture from tetrathionate has been advocated for the isolation of salmonellae from sea water. Fourteen extra positive results were obtained by subculturing the enrichment media at 72 and 96 hours instead of only at 24 and 48 hours (Report 1959).

It will be appreciated that the choice of the optimum subculture time is complicated, varying from author

to author, varying from whether the fluid medium is selective or unselective and varying from enrichment medium to enrichment medium. I have considered at some length the relevant historical data referring to this subject, and will now set out my own results against this background.

(a) Isolation of Salmonella typhi from naturally polluted water

During the course of investigations into the occurrence of cases of typhoid fever in a Welsh valley (Jones 1949; 1952), I had occasion to examine a culvert water for the presence of Salmonella typhi. The samples of water were 100c.c. in volume and were cultured at 37°C. in 100c.c. double strength selenite F. Subcultures were made at both 18 hours and at 24 hours as both these times are usual for subculture from enrichment media. I wished to know if one time had any advantage over the other. The plating media employed were brilliant green MacConkey agar (Wilson and Darling 1918) and Wilson and Blair's medium. The results are given in Table IV :-

TABLE IV

Category	Number in Each Category
Positive at 18 hours Positive at 24 hours	9
Negative at 18 hours Positive at 24 hours	5
Positive at 18 hours Negative at 24 hours	0
Total positive at 18 hours subculture	9
Total positive at 24 hours subculture	14

The numbers, although small, suggested that 24 hours was a better subculture time than 18 hours for the specimens comprising this series. Further experience indicated that

subculture later than 24 hours resulted in a decrease in the number of positive isolations of Salmonella typhi.

(b) Isolation of salmonellae from various sources

It is felt that the results of this investigation are best considered in table form (Table V).

TABLE V

	Cumulative Total Positives obtained by Subculture at:				Method of Culture
	1 Day	2 Days	3 Days	4 Days	
Human Faeces	54	55	-	-	Selenite F at 43°C.
Sewage	37	63	72	80	"
Abattoir Swabs	31	42	47	-	"
Bakery Swabs	14	24	26	29	"
River Water	28	31	31	31	"
Frozen Egg	61	75	81	86	"
Guinea Pig Faeces	14	14	14	16	"
Human Faeces	13	26	-	-	Selenite F at 37°C.
Abattoir Swabs	17	27	34	-	"
River Water	27	27	28	28	"
Frozen Egg	22	25	25	25	"
Guinea Pig Faeces	13	13	15	16	"

The only conclusion that can be drawn is that subculture after 24 hours from selenite F broth materially increases the chance of a positive isolation. This finding is true irrespective of the serotype involved (with the exception of Salmonella typhi), irrespective of the temperature of incubation of the selenite and irrespective of the contaminated material examined. The abattoir swab results are comparable in the two sections of the table as they were paired specimens derived from 54 positive samples.

The advantage of prolonging the incubation time after 24 hours is clear. Is there any advantage of subculture before 24 hours ? In the first series of stools in Table V, incubated at 43°C., the specimens were subcultured at 6 hours and at 18 hours as well as at 24 and 48 hours. Forty-three positives were obtained at 6 hours and 54 at 18 hours. These findings certainly do not suggest that a 6-hour subculture has any advantage in the isolation of salmonellae from stools incubated in selenite F broth at 43°C. On theoretical grounds, I do not see that a 6-hour subculture has much to recommend it in the isolation of small numbers of salmonellae from selenite F broth. The selective agent has the property of increasing the generation time of salmonellae. This can easily be demonstrated by progressively increasing the concentration of selenite in a series of fluid media. The same can be done with tetrathionate. If Leofler's calculations on the multiplication of Salmonella typhi in an unselective fluid medium are correct, I feel that his recommendation that subcultures should not be made before 18 hours is even more valid for fluid media containing selective agents.

There is one final point in connection with multiple subculture which should be mentioned. Occasionally a batch of selenite broth is made which is somewhat inhibitory. Salmonella multiplication is slowed and positive subcultures may not be obtained until 48 hours or later. In such a case, subculture after 24 hours is essential for a positive result.

To summarize: multiple subculture from selenite F broth is valuable in the isolation of salmonellae. It helps to compensate for delayed multiplication of salmonellae which may occur owing to excessive inoculation of the enrichment medium, or which may be due to an inhibitory batch of the selenite F broth. In the next section, I will discuss briefly whether all these four subculture times are necessary.

IV HOW MANY SUBCULTURES SHOULD BE MADE FROM THE ENRICHMENT MEDIUM ?

In the previous section, at attempt was made to demonstrate the advantage of subculturing from an enrichment medium after 24 hours. In this laboratory, I frequently use three or four subcultures from selenite F broth. That is to say, I subculture at 24, 48 and 72 hours, or at 24, 48, 72 and 96 hours. Faecal specimens are usually only subcultured at 24 hours, but human foods, animal feeding stuffs, liquid egg for bakery use, polluted river water, sewage and Moore swabs from bakery drains and abattoir drains are all subcultured three or four times. Not every laboratory will find such a prolonged examination convenient, so that some guide is given in Table VI of the results to be expected from one subculture, combinations of two subcultures and combinations of three subcultures. It appears from the table that the advantage of late subculture is more striking with specimens of sewage, egg and swabs from bakery gullies. It is with such specimens that multiple subculture is of the greatest importance if the fullest bacteriological information is to be abstracted from a single specimen. The abattoir swab results in this table were derived from a study of 54 paired positive samples. It is, therefore, possible to compare the isolations at 37°C. and 43°C. in this particular case.

In Part II of this thesis, an outbreak of food poisoning due to bakers' confectionery is described (Harvey, Price, Davis and Morley-Davies 1961). In this incident, both of the serotypes causing the outbreak could not have been isolated from the suspected food if multiple

TABLE VI

Sample	Numbers of Positive Isolations at Various Subculture Times																	Method of Culture
Days or Day Combinations	1	2	3	4	1 + 2	1 + 3	1 + 4	2 + 3	2 + 4	3 + 4	1 + 2 + 3	1 + 2 + 4	1 + 3 + 4	2 + 3 + 4	1 + 3	+	2 + 4	
Sewage	37	58	61	64	63	70	74	67	73	73	72	78	79	75		80		Selenite F at 43°C.
Abattoir Swabs	31	39	40	-	42	45	-	46	-	-	47	-	-	-		-		"
Bakery Swabs	14	20	17	18	24	23	26	22	25	20	26	29	26	25		29		"
River Water	28	27	25	21	31	31	31	30	30	28	31	31	31	31		31		"
Frozen Egg	61	70	80	83	75	81	85	80	84	85	81	85	86	85		86		"
Guinea Pig Faeces	14	13	12	11	14	14	14	14	14	12	14	14	14	14		16		"
Abattoir Swabs	17	23	24	-	27	29	-	31	-	-	34	-	-	-		-		Selenite F at 37°C.
River Water	27	24	17	11	27	28	27	25	24	18	28	27	28	25		28		"
Frozen Egg	22	25	22	23	25	23	23	25	25	23	25	25	25	25		25		"
Guinea Pig Faeces	13	12	13	11	13	15	15	15	14	14	15	15	16	16		16		"

subculture had not been used. Salmonella thompson only was isolated from the 24-hour subculture, while both Salmonella typhi-murium and Salmonella thompson were easily isolated at the 48-hour subculture. If this technique of examination had not been employed, the mixed infection in the food which corresponded as regards serotype and phage type with the infections in the victims, would undoubtedly have been missed.

V ARE DEOXYCHOLATE CITRATE AGAR AND WILSON AND BLAIR'S BISMUTH SULPHITE AGAR SO MUCH SUPERIOR TO A SIMPLE BRILLIANT GREEN TAUROCHOLATE AGAR THAT ONE CAN DEPEND EXCLUSIVELY ON THEIR USE ?

Introduction: Critical review of selective media

Two alternative groups of selective nutrient agars have been used in the development of media designed for the purpose of isolating salmonellae. Firstly, there are the media incorporating one of the triphenylmethane group of dyes; secondly, the selective agent is a salt of one of the bile acids.

Dye media

The early media of the first group made use of malachite green (Loeffler 1903, 1906, 1907; MacConkey 1908). This dye later gave place to its homologue, brilliant green, the change first being made on empirical grounds (Conradi 1908). Later the substitution gained support from theoretical considerations (Stearn and Stearn 1924; Albert 1942). Brilliant green has persisted in use as a selective agent in the isolation of salmonellae, and many media have been described which depend on it for their effectiveness. The earlier brilliant green media had certain disadvantages. The range within which selectivity was combined with satisfactory growth of the typhoid bacillus was narrow. Different batches of agar with the same quantities of brilliant green produced somewhat different results. Cloudiness of the agar rendered a certain amount of brilliant green inactive. Some of these difficulties were resolved by the combination of eosin and brilliant green (Teague and Clurman 1916), and

differentiation was later improved by the further addition of methylene blue (Knox, Gell and Pollock 1942). The increased complexity of these later formulae brought with them difficulties in standardization, and in 1943 Hoyle recommended an acid fuchsin, brilliant green, lactose, bile salt, agar. This he stated was easy to prepare, was stable, and gave satisfactory results with both typhoid and paratyphoid bacilli. Hoyle's medium was similar to the agar medium containing brilliant green, lactose and taurocholate described earlier by Wilson and Darling (1918). Both formulae were remarkable in that brilliant green was combined with sodium taurocholate, which rendered the dye less toxic to both salmonellae and Bact.coli (Winslow and Dolloff 1922; Bulloch 1929). This combination may, however, have widened the effective range over which selectivity could be obtained in an analogous manner to an eosin, brilliant green medium.

All the formulae quoted are examples of dye indicator media, depending for their differentiation on acid formation by the non-pathogens. A further development of the brilliant green media was the bismuth sulphite, brilliant green agar of Wilson and Blair (1927, 1931). Here use was made of the formation of bismuth sulphide by the pathogen. The chief selective agent in these media was stated to be brilliant green (Tabet 1938). The bismuth sulphite media, under favourable conditions, gave excellent differentiation. They are also useful in the isolation of the arizona paracolon group of bacteria (Harvey and Price 1962).

Bile salt media

The second group of selective agents consisted of the sodium salts of taurocholic and deoxycholic acids. The earliest of the formulae employing this group was MacConkey's medium (MacConkey 1908). This was found to be insufficiently selective for the salmonella group. Its main virtue was to distinguish between lactose and non-lactose fermenters. The most successful of the bile salt media was deoxycholate-citrate agar (Leifson 1935) and its modification by Hynes (1942). This medium was highly selective for shigellae as well as salmonellae, so that it was particularly adapted to routine work. The D.E.C. formula of Panja and Ghosh (1943) combined sodium taurocholate with sodium citrate as selective agents. This medium has rarely been used in this country. My own experience with it has been equivocal.

During the last decade there has been a tendency for bismuth sulphite, brilliant green combinations and deoxycholate citrate agar to displace the older dye indicator, brilliant green, media from favour. The reasons for this neglect are not entirely clear. Neither deoxycholate citrate agar nor the bismuth sulphite media can be described as ideal. Differentiation with deoxycholate is a matter of some difficulty, when pathogens and non-pathogenic non-lactose fermenters have to be distinguished from one another. Increasing use of this medium has led to a multitude of screening procedures. The necessity for introducing such indirect aids to visual differentiation tells its own story. Bismuth sulphite

media may be criticized even more adversely on the grounds that different batches often exhibit a high degree of variability. I have found variation occurring even within the same batch, and this too has been recorded by other workers (Cook 1952). Strains of salmonellae that will not grow on this medium have been encountered (Knox, Gell and Pollock 1942; Williams Smith 1952); and when plates are freshly prepared, growth of such a common organism as Salmonella typhi-murium may be grossly inhibited (Cook 1952). In this connection it is worth mentioning that Holt, Vaughan and Wright (1942) stated that it was inadvisable to use freshly prepared Wilson and Blair's medium. Variation from batch to batch has also been experienced with deoxycholate agar, and this has been related to the narrow range of ingredient variation that is permissible with this medium (Crone 1948). It is not the purpose of this paper to decry either the bismuth sulphite or the deoxycholate media. I would suggest, however, that their performance is not such as to commend them for exclusive use. I agree that both these media are to be preferred to the brilliant green dye indicator media for the isolation of typhoid bacilli, and that the bismuth sulphite media are pre-eminently suitable for this purpose. Where, however, comparisons between a brilliant green medium and either deoxycholate citrate or bismuth sulphite agar have been made for Salmonella paratyphi B, no great degree of superiority has been shown by the latter two media (Wilson and Blair, 1931; Glass and Tabet 1938; Knox, Gell and Pollock 1942; Hoyle 1943).

Experimental work

The aim of this investigation was to compare the performance of a very simple brilliant green medium with deoxycholate citrate agar and Wilson and Blair's medium. The brilliant green medium employed was prepared by adding the dye in a final concentration of 1/30,000 to ordinary MacConkey base pH 7.4. This medium is able to grow Salmonella typhi as well as other salmonellae.

When it was not considered necessary to have a medium capable of growing typhoid bacilli, a higher concentration of dye was used to give a greater degree of selectivity, though concentrations above 1/20,000 occasionally resulted ina diminution in size of the salmonella colonies, and differentiation suffered despite the increase in selectivity.

I have not found it necessary in routine culture to titrate different batches of brilliant green. In brilliant green MacConkey, I considered that I had a medium, simple to prepare from a base kept in any bacteriological laboratory, which depended on a single selective agent of comparatively standard composition. I have examined the range of concentrations of the selective agent necessary to obtain suppression of non-pathogens without unduly preventing the development of the pathogens. This range appeared to be 1/20,000 to 1/50,000 as tested with faeces containing small numbers of salmonellae and from 1/30,000 to 1/50,000 for the culture of Salmonella typhi. There were, therefore, wide limits of permissible variation in the preparation of this medium. The keeping quality was good,

and on one occasion plates left at room temperature for fourteen days unprotected from daylight grew Salmonella typhi well. The plates of brilliant green MacConkey's medium require only 18 - 24 hours' incubation before examination. This is of considerable practical advantage (Dixon 1961).

Results

The three media were compared over a period of three and a half years for stool cultures, and for one year for the culture of sewage swabs. Identical specimens were used for the comparison. There was no weighting of the results with large numbers of stool specimens from a small number of chronic carriers. In stool specimens, Salmonella paratyphi B, Salmonella typhi-murium and other salmonellae were considered separately. With sewer swabs, the results were merely recorded on the basis of whether or not members of the salmonella group were isolated from the samples. The reason for this was that more than one type of salmonella can often be isolated from a single sewer swab. It is by no means easy to decide whether or not a plate showing several hundred suspicious colonies is a pure culture of one salmonella, or whether a minority of colonies exist that belong to a different serological type. The labour required to clarify this point is not always commensurate with the information gained. It is comparatively easy to single out one or two colonies of Salmonella paratyphi B from large numbers of colonies of Salmonella typhi-murium by using the slime layer production by the former. This is a particular case, however, and not

all the colonies of Salmonella paratyphi B on a plate produce a recognizable slime layer, nor is this property specific for Salmonella paratyphi B. It is safer, therefore, to regard all plates yielding colonies of salmonellae from sewage specimens as being potential mixtures of salmonellae. This point will be dealt with later.

The specimens of faeces were cultured immediately on the media to be compared and also for enrichment in selenite F broth (Leifson 1936; Hobbs and Allison 1945a). The latter was subcultured on to the same solid media after 18 - 24 hours' incubation at 37°C. A quantity of broth washings from the sewer swabs was added to an equal amount of double strength selenite, duplicate enrichment cultures being made for each swab. One member of each pair was incubated at 37°C.; the other was incubated at 43°C. in a water-bath. The time of incubation was 24 hours, after which the two enrichment broths were plated on to the three solid selective media. The results of the stool examinations are given in Tables VII and VIII.

These results include all routine specimens, many of which contained very large numbers of pathogens (Thomson 1955). It would not be possible to say that the additional success of the brilliant green MacConkey medium was obtained in the examination of specimens harbouring only small numbers of pathogens, but I suspect that this may have been so. The results obtained with the sewage specimens give some support to this view (Table IX).

In this particular series 16 different salmonellae were isolated, 15 of which were cultures on the brilliant

TABLE VII

Relative Success of Media

Organism	Salmonella paratyphi B		Salmonella typhi-murium		Other Salmonellae	
Method of Culture	Direct	Enrichment	Direct	Enrichment	Direct	Enrichment
Deoxycholate	345 (56)	544 (75)	153 (55)	305 (80)	78 (63)	126 (84)
Brilliant Green MacConkey	563 (91)	605 (83)	247 (89)	349 (91)	111 (90)	138 (92)
Wilson and Blair	447 (72)	538 (74)	166 (60)	266 (70)	92 (75)	115 (77)
Total stools positive direct	618(100)	-	276(100)	-	123(100)	-
Total stools positive by enrichment	-	726(100)	-	382(100)	-	150(100)
Total stools positive by combined examination	848		402		156	

Note that the percentages are calculated on the separate totals of the direct and enrichment examinations, and not on the total positive results obtained by the combined examination, so as to facilitate comparison of the results obtained for different organisms. In Tables VII, VIII and IX, percentages are italicized and enclosed in brackets.

TABLE VIII

Where only One of the Three Media was Successful

Medium	Salmonella paratyphi B		Salmonella typhi-murium		Other Salmonellae	
	Direct	Enrichment	Direct	Enrichment	Direct	Enrichment
Deoxycholate	5 (1)	27 (4)	3 (1)	15 (4)	4 (3)	2 (1)
Brilliant Green MacConkey	99 (16)	57 (8)	58 (21)	28 (7)	12 (10)	6 (4)
Wilson and Blair	42 (7)	65 (9)	23 (8)	12 (3)	8 (7)	8 (5)

TABLE IX

Salmonellae (Including Salmonella paratyphi B)
from Sewage - 64 Positive Specimens

Medium	Enrichment at 37°C.		Enrichment at 43°C.	
	Total Positives	Positive on one Medium only	Total Positives	Positive on one Medium only
Deoxycholate Citrate Agar	16 (25)	0	38 (59)	0
Brilliant Green MacConkey	32 (50)	6 (9)	54 (84)	8 (13)
Wilson and Blair	24 (38)	2 (3)	45 (70)	7 (11)

green MacConkey medium. No Salmonella typhi was isolated. The advantage of culture at 43°C. has already been commented on (Harvey and Thomson 1953) and is not under discussion in the present section. The results are quoted so that the same three solid media may be compared under two different conditions of enrichment.

From these results certain conclusions emerged. Brilliant green MacConkey had the most consistently successful record of the three media for the isolation of salmonellae from faeces and sewage. This result was independent of the type of organism with the exception of Salmonella typhi. From previous observations, brilliant green MacConkey could not be recommended as a single medium for typhoid isolation, but it is worth using in conjunction with Wilson and Blair and Deoxycholate citrate agar. During a regular examination of a culvert water, over twelve months, I never failed to isolate Salmonella typhi on brilliant green MacConkey, although occasional batches of Wilson and Blair's medium gave negative results. The relative position of deoxycholate and Wilson and Blair's as culture media for faeces was in general agreement with the findings of Hobbs and Allison (1945a) and Hynes (1942). The results in this series of examinations of sewage showed that no positive isolations would have been missed if deoxycholate had been omitted.

The figures given go far to suggest that brilliant green MacConkey is a very useful medium for the isolation of salmonellae. Its power to distinguish pathogen from non-pathogen is not above adverse comment (Gray 1929), but

deoxycholate may be similarly criticized. I find secondary plating of a suspicious organism on brilliant green MacConkey agar a most useful test in deciding whether or not it is likely to be a salmonella. I use this procedure frequently when examining isolations from crushed bones, for it is in such material that unusual salmonella serotypes are present. No other selective plating medium is so useful in this respect. The appearance of a fully developed salmonella colony on brilliant green MacConkey is very characteristic to an experienced observer, but as with other media, considerable practice is necessary to get the best out of the medium.

I consider that another reason for the success of the brilliant green MacConkey medium lies in the constancy of its performance. It is a simple medium compared with the other two media. It functions well over a wide range of the selective agent, in contrast to the limited range of permissible ingredient variation possessed by deoxycholate (Crone 1948). Batch-to-batch variability, which occurs with both Tabet's and deoxycholate media (Crone 1948), is rare with brilliant green MacConkey. Variation within the same batch, which is known in Wilson and Blair's medium (Cook 1952), has never been found in my experience of the dye medium.

Over a period of years, I have had to work with several unsatisfactory sets of Wilson and Blair's and deoxycholate plates. The defect in these batches would not have been revealed had brilliant green MacConkey not been available for comparison. In my opinion it is unwise to

depend entirely on two media which have an acknowledged high batch variability. A recent paper by Dixon (1961), confirms the efficacy of brilliant green MacConkey with respect to deoxycholate and Wilson and Blair.

VI IS PRE-INCUBATION OF INFECTED MATERIAL IN AN UNSELECTIVE NUTRIENT FLUID MEDIUM BEFORE SELECTIVE FLUID CULTURE OF ANY VALUE ?

Thomson (1953, 1955) stated that occasionally culture in a medium such as ordinary nutrient broth might have advantages over an enrichment medium. The basis of this statement was an investigation into the survival of Salmonella paratyphi B in flour which had been artificially infected with the organism. Salmonella paratyphi B was recovered from the flour for 45 weeks by means of culture in broth. Cultures of the same flour in selenite F broth were positive up to 21 weeks, but thereafter negative results were obtained. This demonstrated the fact that although Salmonella paratyphi B remained viable in flour for 45 weeks, it was unable to multiply in the relatively toxic environment of selenite F broth, after about 21 weeks. This immediately raises the point that scanty salmonellae in dried foods or materials may be organisms of diminished viability. Such organisms may not be capable of being isolated by the usual enrichment processes, yet may be recovered by culture in plain nutrient broth. Unfortunately culture of a highly contaminated material in nutrient broth is subject to the danger of overgrowth of the scanty salmonellae by other more vigorous organisms. This danger may be obviated by a judicious combination of both cultural techniques - preculture in broth to resuscitate organisms of diminished viability, followed by transference of the entire pre-incubated material to an enrichment medium such as selenite broth of sufficient strength to ensure that the final concentration of sodium hydrogen selenite is 0.4%.

There are several examples of this two stage isolation process in bacteriology. The normal procedure in water bacteriology of culturing water samples in the less selective environment of MacConkey's broth at 37°C. before subculture to MacConkey at 44°C. (a more selective but more inhibitory environment) is a technique that has been accepted for many years. More recently a resuscitative process (Childs and Allen 1953) has been described in which water is cultured for an hour in lactose peptone water at 37°C. without indicator and bile salt. Bile salt and indicator are then added, in such proportions as to convert the basal medium to MacConkey fluid medium. The sample was then incubated at 44°C. for 18 - 24 hours.

Recently Byrne, Rayman and Schneider (1955) stated that direct culture of yeast in selenite F broth yielded negative results, but that a prelminary incubation of a 1 in 10 yeast suspension for 24 hours before subculture to selenite and tetrathionate gave positive salmonella isolations. The fluid in which the yeast was suspended was not stated.

Müller (1952) pre-incubated 1 gm. of bone meal or meat meal in 30 - 40 ml. meat infusion broth before inoculating a part of the resulting growth into mice. Salmonellae were cultured from the mice. Walker (1957), has advocated a preliminary incubation of bone meal in quarter strength Ringer solution prior to adding double strength selenite F.

The technique used for examining dry contaminated samples in this laboratory is to pre-incubate about 50 gm.

of the specimen in about 100 ml. of nutrient broth for 24 hours at 37°C. Then 100 ml. of double strength selenite broth is added and the whole incubated at 43°C. Subcultures are made at 24, 48 and 72 hours onto the selective plating media. No comparative trials have yet been made on the value of pre-incubation however. I hope to do this at a future date when suitable material for study is available.

VII IS A CHANGE IN THE CONCENTRATION OF THE PRIMARY SELECTIVE AGENT IN THE ENRICHMENT MEDIUM DESIRABLE FOR THE ISOLATION OF CERTAIN SALMONELLAE ?

Leifson (1936) in his description of fluid media based on sodium hydrogen selenite made two points which have to a large extent been ignored by later workers. The first was the observation that sodium hydrogen selenite had differential selective properties within the salmonella group. This is best illustrated by reproducing a table from Leifson's original paper. The bacterial nomenclature is as given by Leifson.

Growth of various types of bacteria on infusion agar at pH 7.0 with various concentrations of sodium acid selenite (after Leifson, 1936).

Group I	Growth in 24 hours in a concentration of 1% or over of sodium selenite	(typhoid bacilli (cholera bacilli (variable ?) (pyocyaneus (proteus (paratyphoid bacilli (most but not all)
Group II	Growth in 0.5%, but not in 1% of sodium selenite	(Enterococci (variable) (Aerobacter (Sonne dysentery bacilli (Sal.gallinarum
Group III	Growth in 0.2%, but not in 0.5% of sodium selenite	(Escherichia (Alkaligenes (Serratia marcessens (Pasteurella
Group IV	Growth in 0.1%, but not in 0.2% of sodium selenite	(B.subtilis (Bact.bronchisepticum (Sal.suipestifer (some strains in Group V) (Brucella (some strains)
Group V	No growth in 0.1% of sodium selenite	(Sal.suipestifer (some strains) (Dysentery (Flexner bacilli) (Staphylococci (Sarcina (Brucella (some)

Study of the table indicated that Salmonella

typhi is the most resistant member of the group to the action of selenite and that most, but not all, strains of Salmonella paratyphi B are equally resistant. Certain salmonellae, such as Salmonella cholerae suis are sensitive to selenite and this point has been made much more recently by Williams Smith (1952).

The second important observation made by Leifson was that different materials infected with salmonellae required somewhat different enrichment media formulae for the efficient isolation of the pathogens. Thus, selenite F was devised as the fluid medium most suited for the isolation of salmonellae from faeces and selenite S and selenite M were suggested formulae for infected sewage and milk respectively. Leifson obviously realised that a variation according to circumstances in the concentration of the primary selective agent, i.e., sodium hydrogen selenite was relevant to successful isolation of salmonellae. Yet most subsequent workers confined their attention to selenite F alone. The use of several concentrations of brilliant green in peptone water for enrichment purposes is another illustration of the fact that the amount of selective agent present may determine the result. The use of several strengths of an enriching agent is no longer frequently used in routine salmonella isolation, but Rolfe (1946) does give two separate formulae A and B for tetrathionate which vary in their selective properties. The B formula has considerably more tetrathionate in it than the A. Williams Smith further reduced the concentration of tetrathionate in Rolfe's A formula in order to obtain the

maximum number of isolations of small numbers of salmonellae added to various animal faeces. He also drew attention to the fact that Salmonella cholerae suis would not grow in conventional tetrathionate and selenite broth formulae. For this organism to multiply a further reduction in the quantity of tetrathionate was necessary.

My own personal interest in varying the concentration of the selective agent was to improve isolations of Salmonella typhi from polluted water. I first confirmed Leifson's observation that Salmonella typhi was more resistant to the action of sodium hydrogen selenite than other members of the group. This preliminary work was performed with pure cultures. The next step was to devise a selenite broth with a higher concentration of selenite in it than the 0.4 per cent present in single strength selenite F. A formula with 1.5 per cent was tried, but proved too high. Titration experiments with naturally polluted water suggested that 0.8 per cent of selenite was a satisfactory concentration. This is the percentage present in the double strength selenite F broth which is a usual stock medium to be found in public health laboratories. It is interesting that Clark and his co-workers (1951) have come to the conclusion that Salmonella typhi was better isolated from polluted water by the filtration technique if double strength bismuth sulphite broth was used in place of the conventional single strength formula.

This preliminary work suggested that a trial of single and double strength selenites might be of value

for the examination of water naturally polluted with Salmonella typhi.

Method

Quantities of 100 c.c. of polluted river water were put up in duplicate. One member of the pair was added to 100 c.c. of double strength selenite F broth. The other member of the pair was similarly cultured in four times strength selenite F broth. The respective final concentrations, therefore, of sodium hydrogen selenite were 0.4 per cent and 0.8 per cent. The inoculated enrichment media were incubated at 37°C. for 24 hours and were sub-cultured on to Wilson and Blair's medium. These plates were incubated at 37°C. and were read at 24 and 48 hours. The results were as follows :-

TABLE X

Total Positive Specimens	70
Positive in 0.4% selenite and 0.8% selenite broth	34
Positive in 0.4% selenite and Negative in 0.8% selenite	8
Negative in 0.4% selenite and Positive in 0.8% selenite	28

On a few occasions counts were made of the numbers of Salmonella typhi per millelitre of selenite broth after 24 hours incubation at 37°C. The counts were invariably higher in the 0.8 per cent selenite broth.

The trend is in favour of the 0.8 per cent selenite broth as an enrichment medium for Salmonella typhi. This does not mean that the medium is one of general utility for other salmonellae. A series of experiments with water which was naturally polluted simultaneously with Salmonella

typhi, Salmonella paratyphi B and Salmonella typhi-murium soon indicated that 0.8 per cent selenite had a marked differential selective effect on those three organisms. The growth of Salmonella typhi-murium was completely suppressed while that of Salmonella typhi was encouraged. The effect on the multiplication of Salmonella paratyphi B was variable. Such a differential effect within the salmonella group indicated that 0.8 per cent selenite broth would be unsafe to use as a general enrichment medium for the isolation of salmonellae from polluted waters. I would, therefore, regard Leifson's claims for selenite S (1.5 per cent selenite broth) with some reserve. From Leifson's table with which I introduced this section it is seen that both proteus and pseudomonas are highly tolerant to the action of selenite. This is bourne out by my own experience which suggests that neither of these organisms can be adequately suppressed by increasing the selenite concentration. Yet these organisms are amongst the most troublesome in sewage, owing to their tendency to overgrow salmonellae. It would appear to be more logical to attempt their suppression by adding brilliant green to the selenite broth. Some work on these lines has been performed by Pilsworth (1960).

VIII IN SPECIMENS YIELDING MANY COLONIES OF SALMONELLAE IN PHASE II, CAN THE TECHNIQUE OF PHASE II → PHASE I CHANGE BE IMPROVED ?

The usual method of changing the H antigen of biphasic salmonellae from one phase to another makes use of either the Craigie tube (Craigie 1931; Tulloch 1939) or the Gard technique (1938). There are several variations of the former technique (Hajna 1944; Levine and Preisler 1945; Hinshaw and McNeil 1946; Juenker 1946; Kuhn 1947). In all techniques, semi-solid agar is mixed with antiserum against the H antigen of the culture in the phase which is to be suppressed. On stab inoculation of the treated agar with culture, the homologous phase is immobilized at the site of inoculation, and the alternative phase develops uninhibited and swarms throughout the medium. The phenomenon on which all these techniques depend was originally described by Wassén in 1930.

With the exception of the method advocated by Hinshaw and McNeil, these techniques suffer from certain disadvantages. The semi-solid agar has to be melted and cooled before incorporating the serum, and some of the methods suggested are uneconomical in the use of agglutinating serum.

Examination of crushed bones imported from India showed that almost all samples of this material were infected with salmonellae. Most specimens contained several serotypes and routine examination necessitated the picking of 25 - 50 suspicious colonies from platings of each sample. Many of the salmonellae had their H antigens in Phase II, and a great deal of labour and serum was necessary to

convert these cultures to Phase I for serotype identification. I found orthodox techniques unsuitable and decided to devise a more convenient method for dealing with the problem.

Technique

Bijoux bottles containing 0.15 per cent nutrient agar in 4 ml. amounts were prepared. The brand of agar used was Davis New Zealand Agar. This agar concentration is more fluid than that usually employed in Craigie tubes. Four drops, each 1/50 ml., of the appropriate agglutinating antiserum were added to one or more of these bottles depending on the number of phase changes to be made. The agar and serum were mixed by rotating the bottle in the hands. The agar was sufficiently fluid for this mixing to be accomplished without melting and cooling to 50°C.

A sterile Pasteur pipette (unplugged), kept in a sterilized container, was then filled by a teat with the combination of semi-solid agar and serum to the level of the junction of the stem and barrel. After filling, a small amount of a dense suspension of culture to be examined was carefully sucked up in the terminal 1 cm. of the stem. This suspension had to be in close contact with the agar-serum mixture above it. By manipulating the teat on the barrel end of the pipette, a small amount of air was then sucked up under the suspension of culture, and the end of the stem was sealed in a flame (Figure). The teat was removed, and the pipette was placed in a test-tube with cotton-wool at the bottom and incubated overnight at 37°C. By next morning the unsuppressed phase had grown up the long column of agar, and could be recovered in

FIGURE I

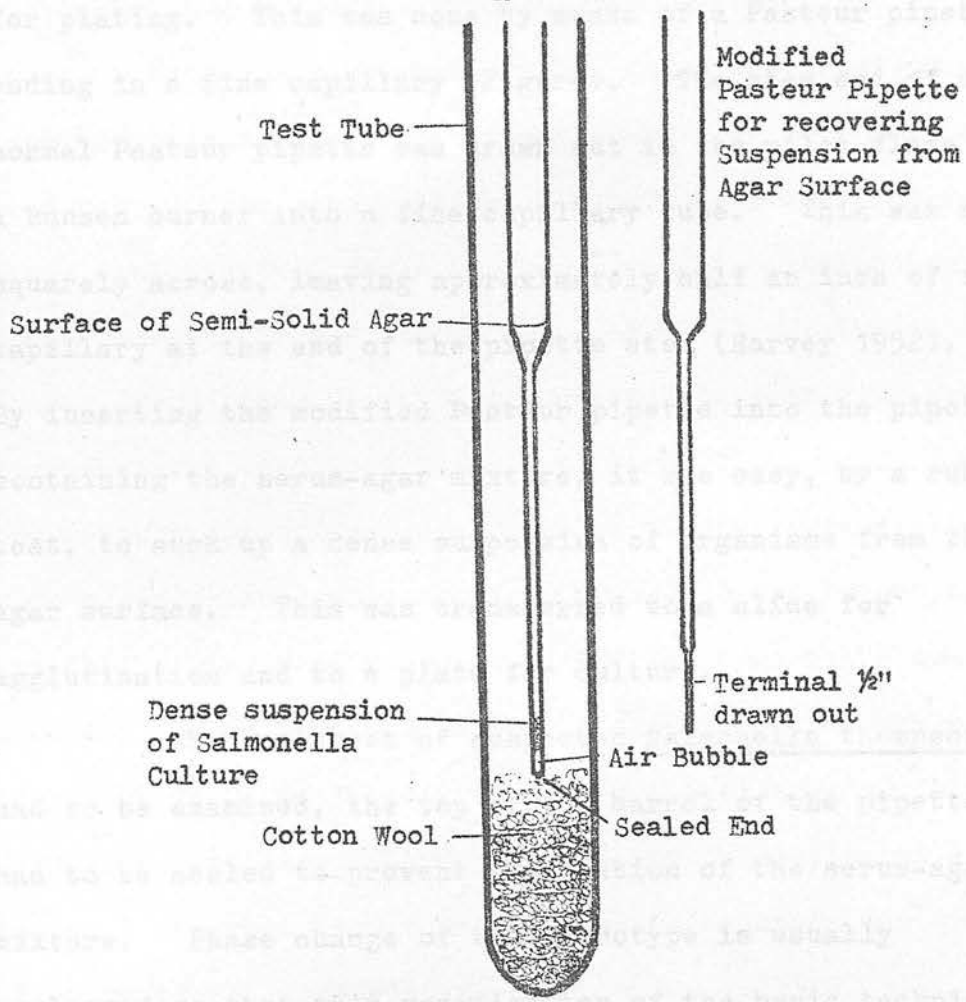


Figure showing the mixture of bacterial suspension and antiserum in a Pasteur pipette in a test-tube, and the modified Pasteur pipette used for recovering the organisms of changed phase from the top of the column of semi-solid agar.

sufficient concentration for direct slide agglutination and for plating. This was done by means of a Pasteur pipette ending in a fine capillary (Figure). The stem end of a normal Pasteur pipette was drawn out in the pilot flame of a Bunsen burner into a fine capillary tube. This was cut squarely across, leaving approximately half an inch of fine capillary at the end of the pipette stem (Harvey 1952). By inserting the modified Pasteur pipette into the pipette containing the serum-agar mixture, it was easy, by a rubber teat, to suck up a dense suspension of organisms from the agar surface. This was transferred to a slide for agglutination and to a plate for culture.

When cultures of suspected Salmonella thompson had to be examined, the top of the barrel of the pipette had to be sealed to prevent evaporation of the serum-agar mixture. Phase change of this serotype is usually prolonged so that this modification of the basic technique was necessary. When the serotype had grown to the upper surface of the serum-agar mixture, the pipette was unsealed by scoring the barrel above the serum-agar surface and pressing a red-hot glass rod against the score. By a prolonged comparative trial it was found that phase change with cultures of Salmonella thompson was accomplished with greater success if the pipettes were incubated at 20°C. and not at 37°C. At 37°C. the growth appearing at the serum-agar surface was very often still in Phase II.

One great advantage of the method was that a bijou bottle of semi-solid agar-serum mixture would keep in a usable condition for about six months in the dark, even in

the heat of summer. This meant that a reagent was available at a moment's notice for most Phase II → Phase I changes encountered. This was a matter of some importance in a laboratory where salmonellae were frequently isolated. When many cultures had to be examined, it was found that about 30 could be changed to the alternative phase by means of one bijou bottle (= 4 ml.) of semi-solid agar-serum mixture. It often happened that it was necessary to change cultures from Phase I to Phase II instead of in the reverse direction. This was particularly important when a single antigenic factor in Phase II determined the identity of the serotype. I have found that one 2 m.m. diameter loopful of Phase I specific serum mixed with ten drops (50 drops to 1ml.) of a 0.15 per cent agar could be used to accomplish this change. Now that single-factor Phase II sera are available to most laboratories the identity of the converted strains can be readily established.

The technique just described has been in use in our laboratory for several years without any difficulty being encountered. The method now forms the basis of examining samples contaminated with several salmonella serotypes. This will be discussed in the next section.

IX WHEN DEALING WITH SPECIMENS INFECTED WITH SEVERAL SALMONELLA SEROTYPES, HAS THE TECHNIQUE OF EXAMINATION A BEARING ON THE NUMBER OF SEROTYPES ISOLATED FROM A SINGLE SPECIMEN ?

The problem in such an examination, is not the simple demonstration of the presence of salmonellae in a specimen, but the isolation of as many serotypes as is technically possible. The methods employed for this purpose use many of the techniques and principles already discussed in the foregoing sections.

The recognition of multiple infection with salmonella serotypes is not new. Conradi (1904), Kayser (1904) and Castellani (1907), have observed patients from whose faeces Salmonella typhi and Salmonella paratyphi B were isolated. A case of triple enteric infection has also been described in which Salmonella typhi, Salmonella paratyphi A and Salmonella paratyphi B were cultured from the patient's stools (Castellani 1915). Apart from multiple enteric infection, there are the associated problems of combined infection with enteric organisms and other food poisoning salmonellae and of multiple infection with salmonellae not associated with enteric fever. For instance, Cernozubov, Filipovic and Stavel (1937), reported 12 cases in which Salmonella typhi and Salmonella enteritidis were found in combination. In 1943, Hormaeche, Surraco, Pelluffo and Aleppo described 32 cases of multiple salmonella infection. Of these 32 cases, 28 were infected with two serotypes, two with three, one with four and one with five. A later case is also described from whom ten different serotypes were isolated over a series of examinations. It is interesting to note that these authors

insisted on the full identification of no fewer than 40 colonies of salmonellae from each patient as a routine procedure. In 1945, Juenker noted the isolation of four salmonella types from one carrier and the same author has recently reviewed the problem of multiple salmonella infection, and has described a technique for examining faeces for multiple serotypes (Juenker 1957).

Multiple salmonella infection of animals is not uncommon and Edwards and Bruner (1940) review some of the relevant literature on this subject, with special reference to salmonella infections of fowls. Multiple salmonella infection of pigs with Salmonella derby and Salmonella typhi-murium has been recently noted by McDonagh and Smith (1958), in pigs after a prolonged stay in the lairage of an abattoir.

There are other specimens apart from those directly derived from man and animals which are subject to multiple contamination. Conradi (1904) examined polluted water which was thought to have caused a double enteric infection in a small girl. From it he isolated Salmonella typhi and Salmonella paratyphi B. Gell, Hobbs and Allison (1945) observed the ease with which Salmonella paratyphi B was isolated from a sewage effluent in contrast to the more difficult recovery of Salmonella typhi. This was noted, although the number of excreters of Salmonella typhi, which were polluting the effluent, numbered eleven, compared with the single excreter of Salmonella paratyphi B. In 1956, I drew attention to the very common occurrence of multiple salmonella contamination of urban sewage specimens

(Harvey 1956). This multiple contamination was of some technical interest as it had occasionally interfered with the isolation of Salmonella paratyphi B in a sewage survey of Cardiff, and had prolonged the time required to trace foci of Paratyphoid infection (Harvey and Phillips 1955). In 1957, I again referred to the presence of several salmonella types in sanitary specimens and suggested techniques, which might be of value in revealing the presence of several serotypes from a single specimen.

Occasionally foodstuffs have to be examined which contain more than one salmonella serotype and this very multiple infection may interfere with the isolation of the type of salmonella it is desired to culture. As an example of this, Wilson and Mackenzie (1955) drew attention to the difficulty they experienced in isolating Salmonella typhi from dessicated coconut which contained other salmonella serotypes. Recent examination of egg products has revealed the occasional multiple contamination of tins of this product (Report 1958). If one is examining a tin of an egg product for a specific salmonella type, it is as well to remember that multiple contamination with other serotypes may interfere considerably with the isolation.

From the above review it will be realised that many specimens are received in a public health laboratory which are potentially infected with several salmonella types. It may occasionally be of epidemiological importance to reveal the maximum number of strains present in the specimen, and it is to this end that some of the following techniques are directed :-

(a) Isolation by differentiation on the solid medium

Certain salmonella species have a characteristic small size of colony compared with the norm. Examples of such species are Salmonella typhi, Salmonella gallinarum and Salmonella pullorum (Stokes and Bayne 1957). I have made use of this difference in size to separate Salmonella typhi from Salmonella paratyphi B and Salmonella pullorum from Salmonella typhi-murium. Salmonella dublin often is isolated on brilliant green MacConkey medium in a small colony form and this characteristic has enabled me to separate Salmonella dublin from Salmonella enteritidis. The colony of the latter organism is in my experience normal in size on brilliant green MacConkey. In view of the serological similarity of these latter two organisms, it would have been difficult to effect a separation on this medium unless this growth character had been recognised. Recent observations on abattoir specimens have shown that the small size of the Salmonella dublin colony is consistently found on the brilliant green medium.

Apart from the salmonellae which are regularly isolated in small colony form, the size of colonies on both brilliant green MacConkey and on Loureiro's medium may be useful in separating individual serotypes from mixtures. For instance, from a plating of a selenite F culture of a batch of pork sausages on brilliant green MacConkey, suspicious colonies were obtained. These colonies were mainly 3 mm. in diameter, smooth and round, but a minority were much larger, polyhedral in shape and with a less smooth surface. The small colonies were all found to be

Salmonella reading, while the large colonies were all Salmonella poona. Different colonial types on Loureiro's medium may sometimes indicate the presence of multiple serotypes. This point has also been noted by Arnold (1956) using a modification of Hobbs, King and Allison's (1945) formula for bismuth sulphite agar. Salmonella paratyphi B is a particular instance of a species which has a characteristic colonial appearance. On many media Salmonella paratyphi B develops a characteristic slime layer at the periphery of its colonies if plates are left at room temperature after 24 hours incubation at 37°C. This feature was noted as early as 1904 by Conradi, but was discussed at greater length by Müller in 1910. The development of a mucoid wall is often a characteristic of Salmonella paratyphi B, but is not a constant property. I have twice isolated strains unable to form a slime layer, one from a sewer, a strain which was dulcite non-fermenting and monophasic, and the second - a biphasic dulcite fermenter - from an open floor gully in a bakehouse. I have also isolated one strain of Salmonella dublin from a slaughter house which produced a good slime layer on further incubation at room temperature. With these provisos, however, the "Schleimwall-Versuch" of Müller (1910) is extremely useful in isolating Salmonella paratyphi B from sewers containing other salmonellae, and, per contra, it is equally useful when one is examining river water containing Salmonella paratyphi B , for other salmonella serotypes. By examining the edge of the confluent growth on a brilliant green MacConkey plate with a good hand lense, the slime

layer is often more easily recognised than around individual colonies. This, on an occasion, allowed one to pick a colony of Salmonella oranienburg from between two colonies of Salmonella paratyphi B. Without the use of the "Schleimwall-Versuch", this specimen would have been regarded as growing a pure culture of Salmonella paratyphi B.

(b) Isolation of several serotypes by multiple colony picking

Where a plate has to be examined, on which there is no recognisable difference between colony and colony, multiple picking has to be resorted to. It has already been noted that Hormaeche et al. (1943) picked and fully identified 40 colonies from each patient as a routine. Juenker (1957) examined from 55 - 489 colonies from each patient in a study of infection with multiple types of salmonellae.

My own observations have convinced me that the number of serotypes which can be isolated from a specimen is undoubtedly a function of the number of colonies picked. For instance, in a plating from a selenite F culture of Indian crushed bone fragments, 50 colonies were isolated and identified. These were as follows :-

13	(26%)	Salmonella oranienburg
11	(22%)	Salmonella anatum
9	(18%)	Salmonella kirkee
10	(20%)	Provisional new type (45:d-enx)
5	(10%)	Provisional new type (45:z_{29})
1	(2%)	Salmonella richmond
1	(2%)	Salmonella bronx

Had not more than the first ten colonies been picked, only four out of seven serotypes would have been identified.

The importance of isolating many serotypes may not be paramount in some specimens. Until 1959 (See Page 106)

I had not found any multiple infections in faecal specimens. In such samples in this country, in contrast to South America (Hormaeche et al. 1943), multiple infections may well be rare. On the other hand, where a survey of salmonella serotypes is being made on an imported material which enters into the composition of animal feeding stuffs, a further examination is obviously desirable. Such a substance is bone meal made from crushed bone fragments of Indian origin. The picking and identification of 50 colonies is not as time consuming as might be imagined. Picks can be made with a straight wire directly from the Loureiro plates and subcultured to the water of condensation at the foot of small agar slopes tubed in bijou bottles. Fifty colonies can be picked in 20 minutes. The inoculum is drawn once up over the agar surface after inoculating the water of condensation. The growth in the condensation water is sufficient after five to six hours incubation in a 37°C. water bath for one of the 'H' antigens to be identified. For the more unusual serotypes, Burroughs Wellcome diagnostic sera are used, as the range of the Standards Laboratory sera is not sufficiently wide. Contrary to expectations it was found perfectly satisfactory to pick colonies direct from Wilson and Blair's agar. From the usual descriptions of this medium, direct picking is inadvisable without plating for purity. This is so, as far as the inoculation of 'sugars' is concerned, but in 500 picks only two were contaminated to an extent that made identification impossible. Intermediate plating of all colonies for purity would

remove this technique outside the limits of a routine laboratory procedure. It is essential to keep a technique within the bounds of a routine examination and I have found the above method fulfils this requirement admirably.

(c) The use of multiple subculture from selenite F broth as a means of isolation of multiple serotypes

I have indicated above that the number of serotypes isolated is governed by the number of colonies picked. The identification of 50 colonies is not necessarily an impossible task in routine practice. Yet the picking of even this number of colonies at a single subculture time may not be able to reveal all the types present. The isolation and identification of fewer colonies from plates inoculated at different subculture times, may often give a more satisfactory result. The labour involved is less and the modified technique may more easily be integrated with routine laboratory examinations. The evolution of this procedure started from a chance discovery some years ago.

In October 1955 a gauze swab, which had been left for 48 hours in a drain in a bakery, was submitted for examination. This swab was one of a series intended to survey salmonella contamination in the environment of the bakery. After incubation in selenite F broth at 43°C., the enrichment medium was subcultured at 24, 48, 72 and 96 hours on to brilliant green MacConkey medium. The plating at 24 hours was negative. The 48-hour plating produced a growth of Salmonella sundsval, while the 96-hour subculture produced a growth of Salmonella thompson. The

former salmonella was predominant at 24 hours, the latter at 96 hours. A specimen from the same drain submitted in January 1956 produced a similar result. In this case, Salmonella thompson only was isolated from the 24- and 48-hour subcultures from selenite F broth while Salmonella aberdeen only was isolated from the 72- and 96- hour subcultures.

Samples of polluted river water and crushed bones have been examined for multiple serotypes by this method of subculture at several times from selenite broth. The method has been highly successful in a survey of Indian crushed bone for Salmonellae and Arizona paracolons (Harvey and Price 1962). One illustrative experiment only need be quoted, although the technique has been shown to be valid for all classes of specimen whether cultured in selenite broth at 37°C. or at 43°C. Naturally infected bones, after an hour's pre-incubation in broth at 37°C., were separated from the supernatant fluid. This fluid was then diluted to 50c.c. with tap water and 50c.c. of double strength selenite F broth was added. The 100c.c. of fluid was incubated at 43°C. Subcultures were made to Wilson and Blair's medium. The subculture times chosen were 5, 18, 24, 48, 72 and 96 hours. Five plates were inoculated at each separate time. The plates were incubated at 37°C. for a full 24 hours and 48 colonies were picked to agar slopes from each positive plate at each subculture time. The subculture at 5 hours was negative, but plates subcultured at later times were all positive. To make up the 48 picks, ten colonies were taken from each of four plates and eight

colonies from the fifth plate. The figure of 48 picks was merely chosen for convenience as the laboratory racks exactly accommodated this number of bijou bottles. The colonies picked were identified serologically and confirmation of the serotype identification was obtained from the nearest salmonella reference laboratory. Six serotypes were isolated. The percentage (of 48 colonies) representing each serotype at the five subculture times is given in Table XI.

TABLE XI

Serotype	Percentage of 48 Colonies Picked at Subculture Times				
	18	24	48	72	96
Salmonella oranienberg	14.6	16.7	4.2	6.3	4.2
Salmonella typhi-murium	56.3	77.1	47.9	8.3	33.3
Salmonella enteritidis (var.jena)	27.1	0	0	0	0
Salmonella derby	0	2.1	29.2	4.2	0
Salmonella reading	2.0	4.1	8.3	81.2	62.5
Salmonella newport	0	0	10.4	0	0

In order to show that the percentage of a serotype was not unduly weighted by any one of the five plates used at each subculture time, the above results are arranged in a different way in Table XII.

It would seem from a consideration of these results that the ratio of one serotype to another varied as a function of the time of subculture.

(d) Serological methods in the isolation of multiple serotypes

On a plate subcultured from selenite F broth, inoculated with material containing several salmonella serotypes, many typical colonies of salmonellae may be

TABLE XII

Plates Positive for Individual Serotypes at Various Subculture Times

Serotype	PLATE NUMBER																			
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
	18 hrs.					24 hrs.					48 hrs.					72 hrs.				
Salmonella oranienberg	+	+	+	+	-	+	+	+	-	+	+	-	-	+	-	-	-	-	+	+
Salmonella typhi-murium	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+
Salmonella enteritidis (jena)	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Salmonella derby	-	-	-	-	-	-	+	-	-	-	+	+	+	+	+	+	+	-	-	-
Salmonella reading	-	+	-	-	-	-	-	-	+	+	+	+	+	-	+	+	+	+	+	+
Salmonella newport	-	-	-	-	-	-	-	-	-	-	-	+	-	+	+	-	-	-	-	-

seen. I have already discussed the multiple picking of colonies to decide approximately the number of serotypes present on such a plate. This method is somewhat tedious and as an alternative the use of agglutinating serum to suppress the motility of one or more serotypes, in order that the growth of small numbers of other salmonella types may be encouraged, is worth considering.

The use of agglutinating serum in the selective isolation of salmonellae is not new. It was tried by Loeffler in 1906 with little success. The use of semi-solid agar by Craigie (1931) as a means of selecting motile salmonella organisms from cultures which were largely non-motile, provided the means whereby serum could be used as a selective agent. The combination of semi-solid agar with "H" agglutinating sera was applied by Gard (1938) and by Tulloch (1939) to the separation of the phases of motile diphasic organisms of the salmonella group. Bailey and Laidley in 1955, extended the method to the separation of individual salmonellae from mixtures by the use of "O" or "H" agglutinating sera in plates of semi-solid agar. Juenker in 1957 using a similar technique, was able to study the problem of multiple salmonella infection in humans. By the use of the same fundamental technique, I was able to separate Salmonella thompson from Salmonella sundsval in a river water sample (Harvey 1957).

One can proceed in one of two ways in the serological separation of one salmonella from another. The first method as used by Juenker (1957) was to identify one serotype and then to pick numerous salmonella-like colonies

to Craigie tubes containing antiserum specific for the flagella antigens of the serotype identified. If no spread through the Craigie tube occurred, it was assumed that all colonies picked were of the same type as that originally determined. Where spread occurred through the semi-solid agar the growth was examined for other antigenic factors. Precautions were taken in this method to avoid the criticism that some of the serotypes isolated were serologically induced, by simultaneous use of the multiple pick method without the use of serum. The results obtained by the two methods were compared. Occasionally it was found, that the Craigie tube plus serum enabled serotypes to be demonstrated which were not represented in the types found by the multiple pick method. It is never entirely certain to what extent such isolations could be criticised as serologically induced variants. This is, unfortunately, a fundamental objection to the method until further work shows that serological induction is seldom produced by short exposure to agglutinating serum.

The second method ignores the danger of inducing artificial variants by the use of serum. It has the advantage of simplicity and rapidity.

First, one must identify one of the serotypes present on a plate in terms of its "O" or "H" antigens - usually the latter. Then an emulsion in a few c.c. of broth is made of the entire growth on one of the selective plates, either from the brilliant green or the Wilson and Blair plate. The medium chosen depends on which plate shows the greatest purity of growth of salmonellae. This

is usually the Wilson and Blair's plate. Sera against the identified specific "H" antigens and against the non-specific antigens are mixed with very soft agar in the proportion of one (2m.m.) loopful of each serum for ten drops of the soft agar. The serum-agar mixture is sucked up into a sterile pasteur pipette, and a little of the salmonella suspension is finally sucked into the end of the pipette so that the suspension and serum-agar mixture are in contact. The inoculated pipette is then placed in a test tube and incubated overnight. The surface growth in the barrel of the pipette is subcultured next day to a brilliant green MacConkey plate and a Wilson and Blair's agar, and these are incubated. The growth on these plates can then be subjected to the same process with appropriate antisera. There is no limit to the number of times the process can be repeated as long as further serotypes can be demonstrated.

(e) Partition of the specimen as an aid to the isolation of multiple serotypes

This method is simply an adaption of the first method of obtaining pure cultures from mixed cultures (Lister 1878). If a litre of river water containing several salmonella serotypes is divided into ten equal parts of 100 ml. and each part is cultured separately in selenite F, it is likely that the serotypes isolated may not be identical in each part cultured. To illustrate this, the result of an actual examination is given. One litre of naturally polluted river water was divided into ten equal parts, and each part was cultured separately for salmonellae. The results are given below :-

Serotypes Isolated

First 100 ml.	Salmonella menston Salmonella paratyphi B
Second 100 ml.	Salmonella chester
Third 100 ml.	Salmonella typhi-murium
Fourth 100 ml.	Salmonella thompson Salmonella chester
Fifth 100 ml.	Salmonella thompson Salmonella paratyphi B
Sixth 100 ml.	Salmonella menston
Seventh 100 ml.	Salmonella chester
Eighth 100 ml.	Negative
Ninth 100 ml.	Negative
Tenth 100 ml.	Negative

The disadvantage of the method is that it is extravagant of media and that the effective size of the separate portions cultured can only be guessed. Nevertheless, the method is worth keeping in mind when water, sewage, Liquid egg or bone meal are being examined for multiple salmonella serotypes. It is also of value for examining polluted river water for multiple phage types of salmonellae.

(f) By suppression of growth of one of the serotypes by physical or chemical means

Not all salmonellae are able to grow at 43°C., although the large majority are able to do so. If a specimen containing Salmonella pullorum and Salmonella typhi-murium is cultured at 37°C. in selenite F, it is possible that both serotypes may appear in the subculture. It is also quite possible that at the 24-hour subculture only Salmonella pullorum will show on plating while the same specimen cultured at 43°C. will show only Salmonella typhi-

murium. It has also been found that the incubation of plates at two temperatures may show differential preferences for cultural temperature in certain salmonella strains. Thus Salmonella thompson failed to grow on a plate of Wilson and Blair's medium incubated at 43°C. while Salmonella paratyphi B grew with ease. The strain of Salmonella thompson grew easily at 37°C. on a duplicate plate of the same medium.

Sodium hydrogen selenite is known to have a differential selective action within the salmonella group (Leifson 1936). If a water supply contaminated with Salmonella typhi and Salmonella typhi-murium is cultured in selenite broth (the term selenite F is purposely not used), in which the concentration of the chemical is raised to 0.8 per cent in place of the usual 0.4 per cent, Salmonella typhi will tend to grow and Salmonella typhi-murium will be suppressed. If the same specimen is cultured in 0.4 per cent selenite - the normal concentration in selenite F broth - Salmonella typhi-murium will probably grow well and may obscure the colonies of Salmonella typhi. Some strains of Salmonella paratyphi B are also selenite tolerant, but not to the same degree as Salmonella typhi. I have used this method in the examination of a water supply regularly containing Salmonella typhi, Salmonella paratyphi B and Salmonella typhi-murium.

In this connection I should remark that in the examination of sewage it has been found inadvisable to use Leifson's selenite S medium which contains 1.6 per cent concentration of sodium hydrogen selenite. This

concentration is too high and may suppress the growth of certain salmonella strains present in the sewage.

X WHAT ENRICHMENT MEDIUM IS MOST SUITABLE FOR ROUTINE SALMONELLA ISOLATION ?

There are three enrichment media which I have found to be efficient in the isolation of salmonellae. These media are :-

1. Selenite broth (Leifson 1936; Hobbs and Allison 1945b)
2. Tetrathionate broth (Knox, Gell and Pollock 1943; Rolfe 1946)
3. Malachite green magnesium sulphate medium of Rappaport, Konforti and Navon (1956)

There have been many comparisons between tetrathionate and selenite broth and the literature is somewhat confusing. It is to be noted that many of the comparisons have been between tetrathionate fluid media containing brilliant green and selenite media without this added selective agent. Such comparisons do not answer the question whether tetrathionate or sodium hydrogen selenite per se is the more efficient agent in isolating salmonellae. A recent article on the culture of salmonellae from sewage indicated that selenite brilliant green broth was more effective in inhibiting the growth of proteus and paracolons than tetrathionate (Bloom, Mack and Mallmann 1958), but the conclusion was made on an analysis of unpaired specimens. The preference of Galton and Quan (1944) for tetrathionate brilliant green bile broth over selenite F can also be criticised from the viewpoint that the conclusions were not drawn from the results of the examination of duplicate specimens. The paper by Williams Smith (1952), however, concluded from the examination of identical specimens, that selenite was slightly superior to tetrathionate in the isolation of small numbers of salmonellae. I do not propose to attempt

to decide, from the rather equivocal literature, the relative merits of tetrathionate and selenite broths. It is better to concentrate on the agreed facts, and from a consideration of these come to a decision which medium is best suited for the purpose of salmonella isolation in a routine laboratory.

Tetrathionate media have the reputation of encouraging the growth of proteus (Cook 1952). They do not have the stability of selenite broth. They are inefficient in the isolation of Salmonella typhi, either from faeces or from sewage (Hobbs and Allison 1945a; Moore 1949).

The malachite magnesium sulphate medium of Rappaport et al. (1956), is a poor means of culturing Salmonella typhi and in my hands it is very inhibitory to the growth of Salmonella dublin. On these grounds it fails to fill the role of a medium for general salmonella culture.

My own observations on these two media have led me to the conclusion that balanced tetrathionate broth with brilliant green added can be a most satisfactory medium for isolating salmonellae from polluted water. The usual formulae have, however, to be radically altered if 43°C. is to be used as incubation temperature. This means that two separate media have to be available, one for use at 37°C. and one at 43°C.

The properties of selenite broth have led me to employ it exclusively for routine salmonella isolation. The important characters of this medium are :-

1. It is stable in concentrated solution

2. Selenite F broth can be used either at 37°C. or at 43°C. without formula modification
3. It is less likely to encourage the growth of members of the proteus group than tetrathionate
4. It is simple to prepare
5. It is effective in the isolation of the large majority of salmonellae (Williams Smith 1952)
6. It can easily be modified by adding measured amounts of stock solutions of certain selective agents. Thus a selenite F broth can easily be converted to a medium with double the quantity of sodium hydrogen selenite by the requisite amount of a 40 per cent stock solution of the chemical. Similarly brilliant green selenite can easily be made by a suitable addition of a stock solution of brilliant green. I have found callibrated dropping pipettes very useful for the rapid preparation of selenite broth modifications.

I feel that for routine culture it is difficult to make any other choice than selenite F broth as an enrichment medium for salmonella isolation.

PART II

In the first section, I considered the various factors which may influence the isolation of salmonellae from a variety of specimens entering a public health laboratory.

It is now necessary to see what practical value a sound bacteriological technique has in investigations on the spread of salmonella infection, both in epidemic and interepidemic periods. Much of the value of the isolation of salmonellae from specimens lies in the survey of the problem that is obtained, and in the information gained as to sources of infection and methods of spread which are relevant to the geographical area under study.

Isolations of salmonella from sewage have been of value in the past in tracing foci of enteric fever (Moore 1948; 1950) and it is this problem which I propose to consider first.

SALMONELLA SURVEYS

I THE SURVIVAL OF SALMONELLA PARATYPHI B IN SEWERS. ITS SIGNIFICANCE IN THE INVESTIGATION OF PARATYPHOID OUTBREAKS

The finding of Salmonella paratyphi B in a sewer is usually regarded as an indication that an excreter lives in the neighbourhood. The possibility that the organism may persist long after the infected person has left the district, or become negative, does not seem to have been sufficiently considered. Some recent investigations in Cardiff have shown that this may be of considerable importance.

Technique

For over a year samples of Cardiff sewage, taken by the sewer swab technique were examined to find unidentified sources of infection.

The swabs were covered with single-strength selenite F broth (Leifson 1936; Hobbs and Allison 1945b) and incubated at 43°C. in a water-bath (Harvey and Thomson 1953). Subcultures were made after 24, 48, 72 and 96 hours' incubation on to whole plates of brilliant green MacConkey medium (Wilson and Darling 1918) and Wilson and Blair's medium. The brilliant green plates were read after 24 hours' incubation and the Wilson and Blair plates after 24 hours' and 48 hours' incubation.

Results

These investigations revealed 11 separate foci of paratyphoid B in the city. Six of these foci yielded organisms of phage type 1, two phage type 3a, and one each of types 3a 1 var.1, 3a 1 var.2, and Taunton respectively.

The predominant type 1 shown by the sewer survey is reflected in the figures for the distribution of types isolated from patients suffering from paratyphoid B during the last 14 years in Cardiff.

During an outbreak of paratyphoid fever spread by cream cakes and due to a strain of phage type 1, specimens of sewage from the affected bakery gave disturbing results, for the samples remained positive long after all the known excreters in the bakery had been removed.

The outbreak began in the first week of March 1954, and by March 6th, 10 cases had been bacteriologically confirmed. In succeeding weeks many more cases were identified in the city, as can be seen in Table XIII.

TABLE XIII

Outbreak of Paratyphi B
March - April, 1954

	1	2	3	4	5	6	7
Week Ending	March 6	March 13	March 20	March 27	April 3	April 10	April 17
Proven new cases	10	52	19	7	4	-	-

In all, 92 cases were identified in Cardiff and all of the 76 primary cases ate cream cakes made by the bakery on February 18, 1954. There was no evidence that patients were infected from the bakery after this date. On March 6th, the bakehouse was visited and inspected. Samples of blood were taken from the staff, and two girls who decorated cakes were found to have weakly positive Widal reactions. The first showed agglutination at 1/25 with both BH and BO suspensions. The second showed agglutination at 1/25 with the BO suspension only. Both

girls were excluded from work for further investigation. Samples of faeces were submitted by all members of the staff, including these two girls. The girl whose Widal was positive 1/25 BO and 1/25 BH was found to be excreting Salmonella paratyphi B (phage type 1). This girl remained positive for a further period of six weeks, and a repeat Widal test ten days after the first was strongly positive - 1/500 BH, 1/50 BO. This evidence favoured the view that she was much more likely to have been a victim of the outbreak than the cause of it.

On April 6th, in the sixth week of the outbreak, when the incident was all but closed, it was decided to sample the sewage from the bakery to eliminate the possibility of undiscovered excreters. I was surprised to find that this sewage was positive for Salmonella paratyphi B (phage type 1). The staff were re-examined, but with negative results, and further specimens of sewage continued to show the presence of Salmonella paratyphi B. It appeared possible that in this case I might be recovering organisms that had been passed some weeks previously and which had survived on the sides of the drain. Accordingly it was decided to follow back the bakery sewer. By this means several positive isolations were obtained from the drains from the female-staff lavatories. The drains from the male-staff lavatories were consistently negative. Each subsequent specimen of sewage was found positive with increasing difficulty and it was surmised that the source of the organisms, inanimate or otherwise, was gradually being eliminated. The sewer serving the female-staff

lavatories remained positive for ten weeks (70 days) after the removal from work of the only excreter identified in the outbreak.

The discovery of Salmonella paratyphi B in the sewer of a large bakery and its continued presence for a period of ten weeks after the removal of all known infected persons raised administrative problems of some magnitude. The question of survival of Salmonella paratyphi B in sewage had now to be decided. By pure chance, I was able to obtain guidance on the matter from another source.

In Cardiff only two cases of infection by Salmonella paratyphi B, phage type 3a, have been identified in the past 14 years and both were in the dock area. During the course of the sewage-swab survey of Cardiff, I had identified a source of Salmonella paratyphi B, phage type 3a, in a single house remote from the docks. This source had been traced back, in a very leisurely fashion over a period of 16 months, from the sewage outfall on the coast. Specimens of blood and faeces were obtained from all members of the family with one exception. The specimens were negative and it was suspected that the member who refused to submit faecal samples was the excreter. This man worked as a ship's chandler in the docks - the only area of Cardiff where cases of phage type 3a were known to have occurred. The family then moved to an entirely different district in Cardiff. Sewage draining from this new locality had been sampled on two occasions in a previous survey and Salmonella paratyphi B, phage type 3a, had not been isolated. When the family had settled down

in the new house the sewage of the house drain was again sampled and Salmonella paratyphi B, phage type 3a, was obtained from the individual house drain. Several houses, including the suspected house, drained into a branch sewer. This branch drain after a short course terminated in the sewer running down the main street. The sewage from the other houses served by the branch drain, which were not suspected of harbouring an excreter, was examined on one occasion with negative results. Soon after I had obtained my first positive sample from the sewer of the new district, the suspected excreter died. This gave me an opportunity to confirm the identity of the source of the infection, and, at the same time, to determine the duration of survival of Salmonella paratyphi B on the drain surface after it had left the human intestine. Had I been aware of the possibility of Salmonella paratyphi B surviving in sewers at the time that the family left their previous house, I could have used the move to determine the duration of survival in the sewer of the old house. The problem of survival, however, was not considered until the family had been four months in their new house. I had, therefore, to use the death of the suspected excreter to give me evidence of Salmonella paratyphi B surviving in the sewer of the new house. The sewage was, therefore, sampled frequently at three points. The first point was the individual house drain of the suspected excreter; the second the main sewer above the entry of the branch drain serving the suspect's house and other adjacent houses; the third was the main sewer at the first manhole below the entry of this branch drain.

The drain serving only the suspected house remained positive for Salmonella paratyphi B, phage type 3a, for 49 days after the death of the suspected excreter. Thereafter, results were negative. The main sewer immediately above the entry of the branch drain was consistently negative on all occasions. The manhole below the entry of the branch drain remained positive for Salmonella paratyphi B, phage type 3a, for 72 days after the suspect's death.

As a precaution the sole remaining occupant of the house was investigated once more serologically and by examination of faeces. The results were again negative.

Further information on the survival of Salmonella paratyphi B in sewers was obtained from an isolation hospital. The hospital admitted a case of paratyphoid fever (phage type Taunton) and this hospital had not harboured a patient infected with this type for three years. The drains serving only the hospital were examined, and Salmonella paratyphi B, phage type Taunton, was isolated for 14 days after the patient's faeces were negative. This result was surprising in view of the fact that the patient's stools were treated with 1/20 carbolic acid for four hours before disposal into the water closet.

Discussion

Some evidence of the persistence of enteric organisms in sewers and in sewage is available from other sources. Wormald (1950) has demonstrated that Salmonella paratyphi B can survive on the surface of a post-mortem room drain for 8 days after necropsy of a case of the disease, despite very energetic antiseptic precautions.

Conflicting reports on the survival of Salmonella paratyphi B in laboratory-stored sewage are available. Gray (1929) could obtain no evidence of any appreciable survival, but Wilson and Blair (1931) demonstrated survival up to 21 days. Snell (1943) believed that the viability of Salmonella paratyphi B in mixed excreta depended on the seasonal temperature. He considered that a survival of two weeks was to be expected at 20 - 22°C. in summer, and of eight to ten weeks at 8 - 10°C. in winter. It must be pointed out, however, that the conditions governing the viability of Salmonella paratyphi B in stored sewage and on the surface of a drain in contact with fresh sewage cannot necessarily be regarded as the same.

The three incidents described here suggest that Salmonella paratyphi B can survive for a long time in sewers. The implications of this are interesting. Firstly, the finding of enteric organisms in a particular sewer does not establish that the source of the organisms is still in the area. Secondly, when the methods of detection of salmonellae in sewage are sufficiently delicate, it is not absolutely necessary to leave swabs in the sewer for long periods, for the surviving organisms present on the sides of the drain can readily be isolated. This refers mainly to the smaller sewers near the origin of the infection. I have successfully used a technique in such cases in which the swab is merely wiped along the drain surface and cultured immediately instead of being left in situ for 48 hours. (Swabs left in the drains were always used to control the results obtained with the "immediate" swabs).

Administratively this latter technique had the advantage that progress was more rapid when attempts were being made to follow a trail leading to an excreter. This could be of importance when tracing a temporary excreter who was an essential epidemiological link in a Salmonella paratyphi B outbreak. It was also considered possible that where the contamination in the sewer was not of very recent origin a wipe-swab might give a more satisfactory sample than a swab left in the sewage for a longer period. Thirdly, in the case of the bakery I was able to demonstrate retrospectively the particular portion of the bakery that had been affected. It was also administratively comforting to be able to show the ultimate clearing of the bakery sewer of infection, although I believe that I was, in this instance, merely measuring the duration of survival of Salmonella paratyphi B on the sides of the drain.

With reference to the search for foci of Salmonella paratyphi B in a community, I would tentatively suggest that, where a sewer produces a rich growth (i.e., a growth which is almost exclusively Salmonella paratyphi B) after exactly 24 hours in selenite F medium, such a finding is likely to start a trail that has a reasonable chance of ending in the discovery of an excreter. Where, with the same technique, only a few colonies of Salmonella paratyphi B can be obtained with difficulty, the possibility should be considered that the organisms are survivors of those passed up to ten weeks previously. Trails depending for their elucidation on the isolation of long-surviving organisms are more difficult to follow to a source than

those which rest on the demonstration of fairly freshly passed pathogens.

In studying outbreaks of Salmonella paratyphi B infection over all parts of the country, a large proportion of such infections are related to bakery products. This has also been the experience locally, as in the 1952 epidemic in South Wales (Cully 1953; Thomson 1953).

As a practical conclusion from the findings in this paper, it was decided to make regular bacteriological examinations of the drains from the large bakeries throughout the city. In order to do this, the managerial staff of bakeries were consulted and they readily agreed to allow drain swabs to be placed on their premises. It was felt that this procedure should provide a reasonable chance of isolating either freshly passed organisms or those which were surviving after contamination during the interval between the placing of the swabs.

Summary

Evidence is presented to support the hypothesis that Salmonella paratyphi B may survive for periods of ten weeks in sewers in the absence of a source maintaining the contamination.

The finding of salmonellae in a sewer does not necessarily imply that the contamination from a human source is continuing.

The use of a swab wiped along the side of the sewer and cultured immediately is suggested as a method of sewage-sampling complementary to the swab which is left several days in the flowing sewage. The wipe-swab has

certain administrative advantages over the swab left down when it is important to find an excreter quickly. It is possible that the "wipe-swab" may provide a more satisfactory sample when surviving organisms are being searched for.

II A SURVEY OF A SINGLE RIVER IN GLAMORGAN FOR EVIDENCE OF SALMONELLA INFECTION

If one confines oneself to the study of human infections, when surveying the local salmonella problem of one's own district, the information obtained will undoubtedly be incomplete. The number of cases of human salmonellosis which come to light even in a large bacteriological laboratory only represents a fraction of the whole. If information is obtained from the Veterinary Investigation Department on animal salmonellosis an important gap in one's knowledge is partially filled, but knowledge of the incidence of subclinical salmonella infection in animals is limited.

In the early work in Glamorgan, on the prevalence of salmonella serotypes, I considered that some information might be obtained by regularly examining samples of the River Taff for salmonellae. The information obtained was considered as likely to supplement that obtained by the study of acute human and animal infections. The river chosen drained a considerable area of the county and received effluents from farms as well as human sewage from towns above Cardiff. It was hoped that an overall picture of local serotype prevalence might be obtained.

Results

These are given in Table XIV.

This river survey merely demonstrated the wide variety of serotypes to be found in the county. It showed the existence of a local salmonella problem, but did little else apart from clarify certain matters concerned with the technique of salmonella isolations.

TABLE XIV

Salmonellae Isolated from River Taff
1956 - 1957

	Serotype	Number of Isolations
1.	Salmonella heidelberg	1
2.	Salmonella panama	2
3.	Salmonella senftenberg	2
4.	Salmonella paratyphi B	34
5.	Salmonella san diego	1
6.	Salmonella typhi	1
7.	Salmonella tennessee	1
8.	Salmonella typhi-murium	2
9.	Salmonella oranienburg	1
10.	Salmonella menston	1
11.	Salmonella anatum	2
12.	Salmonella bredeny	1
13.	Salmonella onderstepoort	1
14.	Salmonella taksony	1
15.	Salmonella reading	1
16.	Salmonella chester	1
17.	Salmonella thompson	1
18.	Salmonella durham	1
19.	Salmonella stanley	1
20.	Salmonella muenchen	1
21.	Salmonella cerro	1

III AN ENVIRONMENTAL SURVEY OF BAKEHOUSES AND ABATTOIRS FOR SALMONELLAE

Outbreaks of salmonella infection are frequently associated with bakehouses, and almost every sizable outbreak of paratyphoid fever in this country in recent years has been spread by bakers' confectionery. It is now known that certain ingredients used in the bakery trade harbour salmonellae, including Salmonella paratyphi B. Outbreaks of salmonella food poisoning are not so frequently associated with butcher meat, but during the course of the investigations reported here, evidence was obtained that some of the less dramatic, but widespread, outbreaks of disease in human beings might be spread by this product. Salmonellae are commonly found in animals, and all unsterilised animal products are likely to be contaminated with these organisms from time to time.

The surveys reported here were begun after an extensive outbreak of paratyphoid B fever, associated with bakers' confectionery in South Wales, because it was then apparent that infection was not carried into the bakehouse by the bakers, but was introduced in infected materials (Culley 1953; Thomson 1953). Since this investigation was begun, imported egg products (Newell 1955; Smith and Hobbs 1955; Newell, Hobbs and Wallace 1955) have been shown frequently to harbour salmonellae, including Salmonella paratyphi B. The surveys were continued, however, in order to get some evidence of frequency of infection of bakehouses by salmonellae in the absence of any outbreak of disease in human beings, as it is of great importance, in the study of epidemic disease, to understand what happens during inter-

epidemic periods. The surveys later produced evidence of infection of the staff in the bakeries and of illness in the general public apparently spread from abattoirs, but this did not alter the fact that it was the primary intention of the investigation to gain insight into the spread of salmonella infection without waiting for the occurrence of clinical human illness.

Materials and Methods

Samples were taken using the Moore's gauze swab technique (Moore 1948). In bakehouses, samples were taken from the sullage drains which received the washings of the bakehouse floors and also from the drains receiving the excreta of the staff. In the bakehouse chosen for this investigation, the two drainage systems were separate, and as both were water trapped, there appeared to be no likelihood of cross contamination by means of rats. It was, therefore, assumed that infection of ingredients and of staff were separately reflected in the isolation of salmonellae from each type of drain. Samples were usually taken at weekly intervals. In addition, specimens were examined from time to time from the staff wash-hand basins and from the surface of several articles of bakehouse machinery.

The abattoir samples were taken from five drains. The first drained blood from pigs, the second blood from cattle and the third the contents of pig gut. The fourth drain sampled blood from cattle and sheep and the fifth drain collected the contents of the beef gut processing department. It will be seen from Table XV that the number of samples examined from Points IV and V were relatively small.

TABLE XV

Drains from which Salmonellae were Isolated in Glamorgan Abattoirs 1957 - 1959

Specimen	I	II	III	IV	V	TOTAL
Number Examined	102	75	81	9	7	274
Number Positive	37	35	31	4	4	111 (40.5%)
Serotypes :-						
Salm.typhi-murium	13	17	15			45
Salm.dublin	5	9	5		1	20
Salm.derby	1	2	1			4
Salm.meleagridis	3	3	1			7
Salm.anatum	2		5			7
Salm.thompson	2	1	3		1	7
Salm.bovis-morbificans	2					2
Salm.muenchen	4					4
Salm.senftenberg	2					2
Salm.enteritidis(jena)		2				2
Salm.enteritidis(danysz)		2	1			3
Salm.enteritidis(chaco)		1				1
Salm.kiambu	1					1
Salm.kentucky	2					2
Salm.abony	1					1
Salm.weltevreden	1					1
Salm.paratyphi B				1		1
Salm.heidelberg				2		2
Salm.agama				1		1
Salm.london					1	1
Salm.infantis					1	1

Key :-

I = Pig Slaughter Drain
II = Cattle Slaughter Drain
III = Pig Gut Processing Drain
IV = Cattle/Sheep Slaughter Drain
V = Beef Gut Processing Drain

The laboratory technique was a simple one gradually developed from past experience (Harvey and Thomson 1953; Harvey and Phillips 1955; Harvey 1956; Harvey 1957). The swabs arrived at the laboratory in wide-mouthed screw-capped jars and were never removed from them. Single strength selenite F broth containing a final concentration of 1 in 10^6 brilliant green was poured on to each swab which was then compressed several times with a sterile glass rod to express its fluid into the enrichment medium. The swab, in its surrounding enrichment medium, was incubated at 43°C. in a water bath, and subcultures were made at 24, 48, 72 and 96 hours on to brilliant green MacConkey agar (Wilson and Darling 1918) and on to Wilson and Blair's bismuth sulphite agar. These plates were incubated at 37°C. and were examined at 24 and 48 hours. Suspicious colonies were picked and examined by direct slide agglutination and polyvalent O salmonella serum and polyvalent H specific and non-specific salmonella serum. Colonies giving positive results with the polyvalent sera were then further investigated. No screening media were used, and in almost every case salmonella colonies could be identified accurately by naked-eye examination checked by slide agglutination. The prolonged incubation of the enrichment media was found to be valuable, not only in the separation of salmonellae from non-pathogens, but also in the separation of one salmonella serotype from another. It was, for instance, sometimes found that one serotype predominated at the 24-hour subculture while a different serotype was dominant in a later subculture (Harvey 1957).

Results - Bakehouse Survey

As was expected, salmonellae were frequently found in the bakehouse (Table XVI). Of the 111 samples taken from the bakehouse floor drains receiving spilt raw materials, 31 were positive, the three common types being Salmonella typhi-murium, Salmonella aberdeen and Salmonella thompson which were found on 8, 12 and 9 occasions respectively. It will be noted also that Salmonella para-typhi B, phage type 1, was found on 2 occasions.

Of the 93 specimens taken from the drains receiving the sewage of the staff, 15 were found positive. Salmonella thompson was never isolated and Salmonella aberdeen only once. Salmonella typhi-murium was found on 8 occasions and Salmonella paratyphi B on 2 occasions.

It was by no means difficult to find salmonellae inside the bakehouse. In addition to their occurrence in the floor gullies, they were found in 3/42 samples from the out-flow water from staff wash-hand basins and from several swabs which had been wiped over the surface of the bakehouse machinery. Salmonella typhi-murium and Salmonella aberdeen were cultured from wash-basins and Salmonella thompson and Salmonella virchow from bakehouse machinery surfaces. Routine cleansing of a cake mixer did not prevent me from isolating Salmonella thompson from its surface subsequently.

It is clear, therefore, that the bakehouse investigated was contaminated with many types of salmonellae. There is no reason to believe that the conditions at the bakery examined or the materials employed were significantly different from other bakeries. In fact, the bakehouse in

TABLE XVI

Isolations of Salmonellae from a Single Bakery 1955 - 1956

	Number of Times Isolated	
	Floor Drains in Bakery Preparation Rooms	Bakery Staff Sewage
Total Swabs Examined	111	93
Total Swabs Positive	31	15
Serotype Isolated :		
Salmonella typhi-murium	8	8
Salmonella aberdeen	12	1
Salmonella thompson	9	0
Salmonella paratyphi B	2	2
Salmonella kentucky	0	2
Salmonella sundsval	1	1
Salmonella ness-ziona	0	1
Salmonella newington	1	0
Total Serotypes Isolated	6	6

question is a large one with a national reputation.

It is suggested, therefore, that salmonellae, including Salmonella paratyphi B, are likely to be very frequently present in bakehouses. Certain kinds of bakers' confectionery are ideal culture media for salmonellae and the potential risk to health would appear to be great. Nevertheless, having regard to the theoretical risk it must be admitted that outbreaks of disease are surprisingly rare. In the area served by this laboratory there is a population of nearly one million with, presumably, several hundreds of bakehouses, yet, in a period of five years, there were only two outbreaks of salmonella food poisoning undoubtedly spread by bakers' confectionery and of these one was a relatively minor affair. Neither of these two outbreaks was associated with the bakehouse in the survey. One of the outbreaks is the subject of a separate communication (Harvey, Price, Davis, Morley-Davies 1961).

Results - Abattoir Survey

Many samples taken from gullies of the abattoirs were found positive, and 21 different types of salmonellae were isolated, as shown in Table XVII. Seventeen of these serotypes occur in animal feeding stuffs. Almost 41 per cent of all samples were positive. The commonest serotypes found were Salmonella typhi-murium and Salmonella dublin. The occurrence of Salmonella typhi-murium was of particular interest because, for the first 20 months of the survey, this organism was never isolated from abattoir swabs, and in the second 16 months it was the type most frequently found. Salmonella typhi-murium was first found in the

TABLE XVII

Monthly Isolations of Salmonella Serotypes from Abattoir Swabs 1957 - 1959

YEAR	1957												1958												1959											
MONTH	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
Serotype :-																																				
Salmonella typhi-murium	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	+	+	+	+	+	+	+	+	+	-	-
Salmonella dublin	+	-	-	-	+	-	+	-	+	-	+	+	+	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Salmonella derby	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Salmonella meleagridis	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+	-
Salmonella anatum	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-
Salmonella thompson	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	+
Salmonella bovis-morbificans	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Salmonella muenchen	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-
Salmonella senftenberg	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
Salmonella enteritidis(jena)	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
Salmonella enteritidis(danysz)	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Salmonella enteritidis(chaco)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Salmonella kiambu	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Salmonella kentucky	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-
Salmonella abony	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
Salmonella weltevreden	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
Salmonella paratyphi B	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
Salmonella heidelberg	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Salmonella agama	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
Salmonella london	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Salmonella infantis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+

gullies of the abattoirs in September 1958, in the 21st month of the survey, as shown in Table XVII. The frequent isolation of salmonellae from abattoirs was less surprising than the frequent isolation from bakehouses. It was thought, however, that the occurrence of salmonellae as contaminants of butcher meat would carry less risk to the human population than similar contamination of bakers' confectionery. I had later to modify this opinion.

In September 1958, when Salmonella typhi-murium was first isolated from the abattoir, after failure to culture this serotype over a period of 20 months, there was a slight rise in the number of human infections due to Salmonella typhi-murium in Glamorgan. From January to August, a total of 14 human cases had been identified. In September there were 8 cases, and in October and November the increase in human infections due to this serotype was even greater (56 in October and 20 in November). These cases were widely scattered with none of the usual vehicles to link them together. It was found that the phage type of Salmonella typhi-murium isolated from the abattoir gulley in October and November and of the strains isolated from the majority of the human infections during the same two months was identical - 1a var.3. This phage type was not isolated once from abattoir swabs during the next 12 months, although many other phage types of Salmonella typhi-murium were isolated from this source.

At the end of 1958 I was informed by the Veterinary Investigation Department in Cardiff that cases

of infection due to Salmonella typhi-murium were occurring in calves on certain farms in Glamorgan with some deaths in the animals infected. Strains of the serotype were obtained, and on three out of five of the infected farms the phage type responsible for the illness was 1a var.3. The phage type was isolated from calves in October, November and December 1958, but never subsequently (Table XVIII).

Routine investigation of the human cases of illness, which were scattered over several local authority areas, failed to incriminate any single foodstuff, bake-house or store. There was, therefore, the suspicion that some incidents of Salmonella typhi-murium infection were caused by eating infected local butcher meat. The case for this was later strengthened by analogy with observations on illness due to other salmonella serotypes. There was, for instance, a tendency for human cases due to Salmonella enteritidis (jena), Salmonella derby, Salmonella heidelberg, Salmonella meleagridis, Salmonella agama and Salmonella muenchen to occur in the local population in the same quarter of the year as corresponding samples from the abattoirs were found positive for these serotypes.

Human and Animal Strains Compared

Salmonella typhi-murium had not been isolated for over a year prior to September 1958 and was regularly isolated from abattoir swabs during 1959. It seemed profitable, therefore, to compare the phage types of these strains with those of cultures isolated from human subjects during the same period. Prior to the use of phage typing

TABLE XVIII

Phage Types of Salmonella typhi-murium Isolated each Month 1958 - 1959

Month Isolated	* Cultures from Diseased Animals	Abattoir Swab Cultures	Human Infection Cultures
1958:-			
October	1a var.1 1a var.3	1a var.3	1a var.1 1a var.3
November	1a var.3	1a var.3	1a var.3 1a
December	1a var.3		1a var.1
1959:-			
January	Untypable		1a var.3
February		Untypable	1a var.3
March		Untypable	Untypable
April		1b	Untypable
May		1a; Untypable	1a; 24
June		2b; 1a var.2	1a; U29 Untypable
July	1a var.2	1a var.2 1b; U15	U29; 32 24
August		1a var.2 1a; U30 Untypable	1a; 32 1a var.1
September	1	2a; 2b 2c; 1b	1; 1a 1a var.3 2b; 2c 2; 1a var.1
October	12	1 var.5; U15 Untypable	U59; 2c 1var.5
November			1 var.5
December	12		1a; 2c

* From Veterinary Investigation Department

it was almost impossible to trace the origin of sporadic human infections with Salmonella typhi-murium. The phage typing scheme for this organism, introduced by Felix and Callow (1943, 1951) and Felix (1956), was extended by Callow (1959), and the scale on which the typing of Salmonella typhi-murium of human and animal origin is carried out has recently been greatly increased. As the result of this work it is now possible to compare the distribution of phage types in man with that concurrently prevalent in livestock (Anderson 1960). It has been shown, for example, that types occurring in cattle can be related to outbreaks of human infection. Such studies have shown that diffusely scattered, apparently sporadic cases of Salmonella typhi-murium infection, can be connected with each other and with the sources of their infection. It was thus hoped, that, by parallel observations on abattoir and human isolations, some light might be shed on what proportion of such seemingly sporadic infection might be due to local contaminated butcher meat products. Representative cultures of Salmonella typhi-murium from human infections and from abattoir swabs were, therefore, sent to the Central Enteric Reference Laboratory, Colindale, for phage typing from October 1958, until the end of 1959. A few strains provided by the Veterinary Investigation Department were included. The results are given in Tables XVIII and XIX.

It will be seen that the phage types of Salmonella typhi-murium isolated from the slaughter house swabs corresponded in type, and not infrequently in timing,

TABLE XIX

Distribution of Phage Types of Salmonella typhi-murium in Diseased Animals and Abattoir Drains in Glamorgan in 1958 - 1959

Phage Type	Pig Faeces	Cattle Faeces	Pig Slaughter Drain	Cattle Slaughter Drain
1		+		
1 var.5			+	+
1a var.1		+		
1a var.2	+	+	+	+
1a var.3		+	+	+
1b				+
12	+			
2a			+	
2b			+	+
2c			+	
U15			+	+
U30			+	
Untypable		+	+	+

TABLE XX

Miscellaneous Specimens of Animal Origin Examined for Salmonellae

Specimen	Total Samples	Number Positive	Serotypes Isolated
Pig Spleen	22	0	-
Pig Liver	20	0	-
Pig Blood	7	0	-
Pig Bile	30	0	-
Pork Sausage	84	1	Salmonella poona Salmonella reading
Ox Spleen	21	0	-
Ox Liver	21	0	-
Ox Blood	10	0	-

with the phage types isolated both from animals and from human infections. It was noted that of the 30 typable human strains of Salmonella typhi-murium, 23 (76.7 per cent) belonged to phage types found in the abattoir environment, or in local farm animals. If I exclude strains isolated from definite outbreaks, i.e., phage type 1a var.3 from October 1958 to February 1960, and phage type 2c in December 1960, the remaining 25 typable cultures represent sporadic incidents. Of these, 18 (72 per cent) were types found in the abattoir swabs and in the faeces of local farm animals. The simplest explanation of this finding is that many of the sporadic incidents are associated with eating infected local meat products.

It is the experience of most laboratories that, apart from dramatic outbreaks of food poisoning from one

small identifiable source, there occur, from time to time, incidents of disease not apparently coming from one source, but of a more diffuse nature. It is suggested that these might be associated with contaminated butcher meat.

It has been established very recently (Hobbs and Wilson 1959) that imported carcass meat can be contaminated with salmonellae. It has been shown also that fresh pork sausages can harbour salmonellae (Galton, Lowery and Hardy 1954). In order to see how frequently fresh meat in the abattoir described here was contaminated by salmonellae a limited number of specimens of pork sausage, pig bile, pig blood, pig spleen, pig liver, cattle spleen, cattle liver and cattle blood were examined. The results of these specimens are given in Table XX. In all 215 specimens were examined with only one positive.

Discussion

Samples taken from the floor gullies of bakehouses showed 31/111 (27.9 per cent) to be positive for salmonellae, and having regard to the potential risk it is surprising that outbreaks of food poisoning carried by bakers' confectionery are not more common. Yet, in a period of five years, in the county of Glamorgan, I have encountered only two incidents of food poisoning due to salmonellae which can definitely be said to have been caused by eating bakers' confectionery. Both of these occurred in 1959.

I have found the sewer swab technique an effective means of surveying the environment of food premises for salmonellae. In the abattoir it was more

effective in the demonstration of the presence of salmonellae in the environment than was the examination of meat specimens. This is probably due to the size of sample represented by a single sewer swab. This sampling technique also probably accounted for some of the more unusual serotypes isolated.

The discovery of Salmonella paratyphi B, phage untypable, in an abattoir drain, not subject to human contamination raises the question of occasional incidents of paratyphoid B fever carried by meat. This question has been discussed previously (Medical Officer, Annotation 1951; Nicol 1956; McCann and Cross 1956; Bernstein 1958).

The occurrence of salmonellae in abattoirs was less surprising than their isolation from a bakehouse environment. It was not known, however, what part was played by infection of butchers' meat in the dissemination of salmonellae amongst human beings. A close inspection of the results obtained in abattoirs and the occurrence of human disease produced circumstantial evidence that some of the less dramatic incidents of salmonella infection were indeed spread by butchers' meat. A similar conclusion was reached by McDonagh and Smith (1958). It was thought that rats played little or no part in these findings. Over 200 rats caught outside the abattoir were examined with entirely negative results. It is not, therefore, probable that salmonellae were introduced into the abattoir by rats from outside. I would consider that my isolations of salmonellae from the slaughter house drains reflected infection introduced by farm animals entering the abattoir for slaughter.

The observation that 16 per cent of specimens from bakery staff sewage were positive was of interest. This figure reflected the degree of potential infection of personnel in a trade dealing with raw materials very frequently contaminated with salmonellae. This point has recently been raised (Lancet, Leading Article 1958). My results, however, led me to the conclusion that bakery staff did not excrete salmonellae for a prolonged period, as the staff sewers were only very intermittently positive. This observation was important, as it suggested that only relatively small numbers of salmonellae were being excreted into the sewers and contrasted with results previously obtained when dealing with human cases of frank disease (Harvey and Phillips 1955). The suggestion that the personnel of certain trades including bakeries should be regularly examined for excretion of salmonellae does not, therefore, promise to be a profitable method for the discovery or control of infection. The danger in the bakehouse arises from the entry of infected materials and not primarily from the staff. There is indeed evidence that bakers themselves acquire infection from these materials. McCullough and Eisele (1951a,b), by human feeding experiments with salmonellae, suggested that the infection rate and the duration of excretion was a function of doseage, small doses of the pathogens giving rise to short periods of excretion. It seems possible that the type of infection that occurs in bakery staff, who are probably subjected to frequent contact with salmonellae in small doseage, is of this order. Newell, McClarin,

Murdock, MacDonald and Hutchinson (1959) have drawn a similar analogy in pigs excreting salmonellae. It was interesting to contrast the serotypes found in bakeries with those discovered in abattoirs. The only type commonly found in both environments was Salmonella typhimurium. This was not surprising, as this serotype, more than any other species of salmonella, is a universal pathogen. It is common to egg products, farm animals and animal feeding stuffs. The remaining bakery serotypes were mostly those found in Chinese frozen egg, a material in daily use in the bakery during the major part of the survey. Salmonella kentucky and Salmonella ness-ziona have not been found in Chinese egg and their origin may, therefore, be different. It is possible, that other contaminated raw materials, apart from egg, introduce salmonellae into bakeries.

The contamination of the bakery environment was easy to account for by the amount of egg visible on floors, tables, etc. Some of the staff were covered in egg from hand to elbow, so that the isolations from wash-hand basins were understandable.

The wide range of salmonella species found in the abattoir would lend support to the hypothesis that farm animals become infected by means of animal feeding stuffs. These materials contain a very wide range of serotypes and 17 out of 21 species found in the abattoir are known to occur in animal foods, or in the raw material incorporated in them.

I feel that the surveys described above serve to illustrate certain local and remote aspects of the

salmonella problem. Unfortunately, the solution of this problem is as yet far from being reached.

Summary

Moore's gauze swabs have, in my hands, been found to be a reliable instrument forthe survey of salmonella infection in the environment of two food premises.

A large bakery was demonstrated to be regularly contaminated with salmonellae. Thirty-one floor gully swabs out of 111 (27.9 per cent) were positive for salmonellae. Little spread of infection to the general public was experienced.

Staff infection with salmonellae was shown to occur in this bakery. Fifteen positive specimens of staff sewage out of 93 were obtained (16.1 per cent). There was little evidence of prolonged contamination of the sewers with salmonellae. Staff infections were probably, therefore, of short duration. The staff probably became infected from contaminated goods in the bakehouse. Rat contamination of sewers was excluded by water trapping.

It is not thought that regular examination of staff excreta would be a profitable means of discovering carriers or of preventing contamination of the products. The frequency with which such examinations would have to be performed to discover short term excreters would be administratively impossible.

Of 274 swabs from gullies in abattoirs, 111 were positive for salmonellae (40.5 per cent). Twenty-one different serotypes were isolated. Seventeen of these serotypes are known to occur in animal feeding stuffs.

Parallel examination of abattoir swabs and excreta from human infections for Salmonella typhi-murium, coupled with phage typing of the strains, provided information of the geographically local nature of much sporadic illness due to this serotype. Of a total of 30 typable strains, 23 belonged to phage types found in the abattoirs, or on local farms. Sporadic incidents were represented by 25 of these cultures, 18 of which corresponded in phage type to those found in the abattoirs or in local farm animals. The phage types of Salmonella typhi-murium isolated from the slaughter houses not infrequently corresponded in timing with the strains isolated from human infections.

IV AN OUTBREAK OF SALMONELLA FOOD POISONING ATTRIBUTED TO BAKERS' CONFECTIONERY

Elsewhere (Harvey and Phillips 1955; Harvey and Phillips 1961), the point has been stressed that salmonellae are regularly introduced into bakehouses without any spread of infection to the general public who consume the bakery products. During 1955 - 1958, in Glamorgan, there was no major incident of salmonella food poisoning spread by bakers' confectionery, despite the fact that infected raw materials were found in regular use in bakeries, and that in our previous survey 27.9 per cent of all samples from floor gullies of the bakeries yielded salmonellae. The incident described here, therefore, serves as a contrast to the previous paper (Harvey and Phillips 1961) and records an outbreak of the classical kind. One other small incident was also seen in the summer of 1959 involving similar items of bakers' confectionery, but these two incidents were the first to occur in a period of five years.

The Outbreak

On 10th December 1959, in the afternoon, approximately 270 children of a junior school attended a school Christmas party. On Friday, 11th December, only 219 children attended school and of these many were taken ill during the morning with vomiting and diarrhoea. By the afternoon the school attendance was reduced to 163 and several of the remaining children had to be sent home owing to sickness. The food consumed at the party consisted of bakers' confectionery from a single bakery and luncheon meat sandwiches. Remnants of both types of food eaten were obtained.

Some items of confectionery from the party had been taken home and the total number at risk was approximately 280. In all, there were 209 persons with positive faecal swabs. Fourteen persons who ate trifle from the party at home produced positive faecal specimens. The incubation period ranged from 8 to 78 hours with an average duration of 26 hours.

Investigation of the Outbreak

It was found that the majority of persons who were taken ill after the party had eaten bakers' confectionery. Several persons who had eaten confectionery brought back from the party also became ill. It was, therefore, natural to start investigations at the bakery. Suspicion fell upon a trifle consisting of cake, crumbled by hand, set in jelly, and topped with custard. The only item of the trifle containing egg was the cake. The trifles were sold in individual paper cartons and at least one trifle was allowed for each child. The jelly used was poured on to the cake crumbs at a high temperature.

Faecal swabs were taken from the bakery staff, and Moore's gauze swabs were inserted in the bakery sewers and in a drain receiving rinsings from the washings of bakehouse utensils. Faecal swabs were also taken from the staff in the grocer's shops which provided the luncheon meat.

Bacteriological Technique

Faecal specimens were plated direct on to brilliant green MacConkey agar and were also inoculated into selenite F broth. The direct plates and enrichment

cultures were incubated at 37°C. for 24 hours. The enrichment media were then subcultured to brilliant green MacConkey agar plates, which were incubated at 37°C. for 24 hours. Suspicious colonies were picked from platings and examined in the usual manner.

Sewage specimens and the food remnants were inoculated into single strength selenite F broth. These enrichment cultures were incubated at 43°C. and were subcultured at 24, 48, 72 and 96 hours on to brilliant green MacConkey plates and on to plates of de Loureiro's modification of Wilson and Blair's medium. These plates were incubated at 37°C. and examined after 24 and 48 hours incubation. It is interesting to note, that the double infection of the trifle would have been missed if only 24-hours subculture had been performed. The 24-hour subculture produced colonies of Salmonella thompson only, while colonies of Salmonella typhi-murium and Salmonella thompson were obtained from the 48-hour subculture (See Part I, Section IX).

All the cultures of Salmonella thompson isolated were examined by the phase change method described in Part I, Section VIII.

Results

Salmonella typhi-murium, phage type 2c, and Salmonella thompson, phage type 4, were isolated from the remnants of trifles eaten at the party. No salmonellae were cultured from the luncheon meat. Salmonella typhi-murium, phage type 2c, was isolated from four of the bakery staff, two of whom had had symptoms, and from the two drain

swab specimens. From faecal specimens of the patients, Salmonella typhi-murium was isolated from 117, Salmonella thompson from 23, and both serotypes were isolated from 69. Representative cultures from these specimens showed the type of Salmonella typhi-murium to be 2c, and that of Salmonella thompson to be type 4. These particular phage types are common in egg products. Salmonella typhi-murium, phage type 2c, has not hitherto been common, however, either in abattoir swabs or in human infections in the past 15 months in Glamorgan, and the only culture of Salmonella thompson recently isolated from an abattoir swab belonged to phage type 1. The girl in the bakery mainly concerned with the manufacture of trifles did not produce a positive stool for salmonellae, although she was one of the staff giving a history of diarrhoea before the outbreak. On the other hand, the girl in the grocery shop, who had handled the luncheon meat eaten at the party, produced a stool positive for Salmonella typhi-murium. Further stools from the girl were also positive.

Discussion

Although the isolation of one of the infecting organisms from the girl in the grocer's shop confuses the epidemiological picture, it is nevertheless true to say that the main concentration of infection lay in the bakery. Four bakery staff were excreting Salmonella typhi-murium, both bakery drain swab samples were positive for Salmonella typhi-murium; there was a double salmonella infection in the trifle eaten; and the identical double infection occurred in many of the victims of the outbreak. Further,

the phage types of the infecting serotypes were common in egg products and uncommon in our local abattoir survey. I feel that this evidence points strongly to the bakery as the source of this outbreak.

The method of infection is in doubt. If the cake base of the trifle was infected, the question arises whether the addition of jelly at a high temperature would have sterilized it. A laboratory experiment demonstrated that it was possible to isolate Salmonella typhi-murium from infected cake crumbs even after pouring jelly at a temperature near 100°C. over them. It is noteworthy that some of the trifles prepared for the party were 30 hours old before they were eaten, thus allowing ample time for bacterial multiplication to occur. The work of Heller and Salter (1958) would make it unlikely that salmonellae survived the baking process. It is not possible, or profitable, from the evidence available to suggest the means whereby the bakers' confectionery became infected, but elsewhere Harvey and Phillips (1961) have indicated that various salmonellae, depending on the serotypes contaminating some of the essential ingredients used by confectionery bakers, are so frequently to be found in bakehouses that there is almost constant opportunity for infection of this type of product.

Summary

An outbreak of food poisoning due to Salmonella typhi-murium and Salmonella thompson is described.

These same two serotypes were found in trifle eaten by the victims.

Salmonella typhi-murium was found in the stools of four members of the bakery staff and in the stool of one member of the grocery staff handling luncheon meat eaten by those affected.

Salmonella typhi-murium was isolated from drains in the bakery.

The phage type of Salmonella typhi-murium found in the food handlers, drains, trifle and victims of the outbreak was the same.

The phage type of Salmonella thompson found in the trifle was identical with that found in the victims of the outbreak.

The phage type of both salmonellae suggestedan egg product source.

It is surprising how infequent these outbreaks are, having regard to the very frequent opportunity for the contamination of confectionery products in bakehouses.

V SALMONELLA SEROTYPES AND ARIZONA PARACOLONS ISOLATED FROM INDIAN CRUSHED BONE

The discovery by Walker (1957) in this country and by continental workers (Müller 1952, 1957; Richter 1956; Rohde and Bischoff 1956; Thal, Rutqvist and Holmqvist 1957), that organic materials used in the preparation of animal feeding stuffs were frequently contaminated with salmonellae prompted a similar investigation in Cardiff on crushed bone imported from India and Pakistan. This material had been the subject of investigations on Anthrax in South Wales (Davies and Harvey 1953, 1955), but it had never previously been suspected as a source of salmonella infection. As these crushed bones were used in the preparation of animal feeding stuffs in our area, it was thought profitable to make a detailed survey of the salmonella serotypes that might be present in the material. Arrangements were made to collect samples of the crushed bones at Cardiff Docks from the cargoes as they arrived. In all, 57 samples were examined for salmonellae.

Technique of Examination

Each sample was approximately 50 gm. in weight; three techniques were used :-

(a) Multiple subculture from selenite F broth and picking of eight suspicious colonies from each subculture plate. The rationale of this technique was that, in an enrichment medium inoculated with a mixture of salmonellae, different serotypes may be dominant at different subculture times (Harvey 1957).

(b) Removal, in progressive steps, of serotypes from

the mixture of salmonellae present in a specimen by serological means, thus allowing serotypes present in small numbers to be revealed. The method involved a modification of the phase change technique described by Harvey and Price (1961). Basically the technique was similar to those used by Bailey and Laidley (1955) and by Juenker (1957). It differed, however, in one important aspect. The whole growth from a de Loureiro plate, subcultured from selenite F broth at 24 hours, formed the material examined for multiple serotypes. A thick suspension of the entire growth was prepared in a few ml. of nutrient broth and this suspension was progressively treated with agglutinating sera to remove salmonella serotypes from the population in an orderly and premeditated manner. Both Standards sera and Wellcome brand Salmonella diagnostic sera were necessary to cover the wide range of serotypes encountered. When serotypes were thought to occur in the H phase described by Taylor, Lee, Edwards and Ramsey (1960), it was necessary to use an O serum to remove the serotype. O sera were not, however, used in the technique unless it was thought to be absolutely necessary, as they could not be relied on to secure complete removal of a serotype from the population. With homologous H sera, on the other hand, the immobilization and removal of a serotype was usually complete.

(c) Distribution of the specimen between different volumes of selenite F enrichment broth.

In all three techniques selenite F broth was used exclusively as enrichment medium and 43°C. as incubation temperature for the enrichment process (Harvey and Thomson

1953). The optimum plating media were found to be Wilson and Blair's medium and brilliant green MacConkey medium (Wilson and Darling 1918; Harvey 1956).

The technique necessitated the picking of many suspicious colonies, varying from 32 - 617.

Results

Fifty-six out of 57 samples were positive for salmonellae (98.2 per cent). Fifty-six salmonella serotypes and eight Arizona paracolon strains were isolated. The serotypes found are given in Tables XXI and XXII, with the number of occasions on which each serotype was isolated. The number of serotypes isolated per specimen is given in Tables XXIII and XXIV. Five previously undescribed salmonella serotypes were found. The Arizona paracolons were always found in conjunction with salmonellae.

Two salmonella serotypes could not be completely identified. One of these (40:1v -) did not produce an alternative phase, even when put through a Craigie tube with 1v serum incorporated in the soft agar. The other, 3,10:z_{45}, was thought to be an organism occurring in the H phase described by Taylor, Lee, Edwards and Ramsey (1960). Culture in soft agar containing z_{45} serum did not, however, produce another H phase. These two organisms had, therefore, to be classed as Salmonella unidentified.

Discussion

Examination of Table XXI shows that 38 of the serotypes found in Indian crushed bone are known to have been responsible for cases of food poisoning in this country since 1955. Eighteen serotypes found in this material

TABLE XXI

Salmonella Serotypes Isolated

Salmonella cubana	15	Salmonella grumpensis	2
Salmonella senftenberg	14	Salmonella jodhpur	2*
Salmonella newport	12	Salmonella kandla	2*
Salmonella anatum	11	Salmonella san-diego	2
Salmonella reading	11	Salmonella seigburg	2
Salmonella gaminara	10	Salmonella tel-hashomer	2
Salmonella poona	9	Salmonella treforest	2*
Salmonella oranienburg	8	Salmonella unidentified	2
Salmonella butantan	7	Salmonella adamstown	1*
Salmonella tennessee	7	Salmonella adelaide	1
Salmonella westhampton	7	Salmonella bareilly	1
Salmonella cerro	6	Salmonella champaign	1
Salmonella give	6	Salmonella derby	1
Salmonella hvittingfoss	6	Salmonella dublin	1
Salmonella richmond	6	Salmonella godesberg	1
Salmonella typhi-murium	6	Salmonella huvudsta	1
Salmonella kentucky	5	Salmonella karachi	1*
Salmonella london	4	Salmonella kirkee	1
Salmonella saint-paul	4	Salmonella marylebone	1
Salmonella worthington	4	Salmonella matopeni	1
Salmonella bredeney	3	Salmonella meleagridis	1
Salmonella bronx	3	Salmonella montevideo	1
Salmonella enteritidis	3	Salmonella muenchen	1
Salmonella minnesota (jena)	3	Salmonella newington	1
Salmonella onderstepoort	3	Salmonella pomona	1
Salmonella alachua	2	Salmonella schwartzengrund	1
Salmonella bere	2	Salmonella taksony	1
Salmonella chester	2	Salmonella waycross	1

* New serotype

TABLE XXII

Arizona Serotypes Isolated

9 a,c	:	29 - 31
16	:	22 - 31
20	:	24 - 28 *
26	:	23 - 30
26	:	23 - 21
26	:	26 - 25 *
29	:	33 - 21
30	:	27 - 28

* New serotype

TABLE XXIII

Number of Salmonella Serotypes Isolated per Specimen	Number of Specimens
0	1
1	11
2	14
3	7
4	10
5	3
6	4
7	3
8	0
9	1
10	0
11	0
12	0
13	0
14	0
15	2
16	0
17	1
Total Specimens	57

TABLE XXIV

Number of Arizona Serotypes Isolated per Specimen	Number of Specimens
1	3
2	1
3	0
4	1

have never been isolated in the United Kingdom from human sources in the period 1955 to the present date. Many of the serotypes in Table XXI have been isolated by bacteriologists in India and the part played by them in animal and human infection has been recently discussed (Ganguli 1958; Sharma and Singh 1961; Agarwal 1962; Khera 1962).

The significance of crushed Indian bones in the epidemiology of salmonella infection is difficult to judge, but the fact that 98.2 per cent of samples were found to be infected is a measure of the potential danger of the material, and the quantitative estimates of the numbers of salmonellae in Pakistan bone-meal have been shown to be high (Williams Smith 1960).

The isolation of eight Arizona paracolon serotypes is of interest; it suggests that the epidemiology of disease, due to this group of organisms, may be similar to that of salmonella infection. These organisms are technically of some importance, since they can very easily be picked in error for salmonellae from Wilson and Blair's plates. Many of the Arizona types isolated agglutinated with O or H salmonella sera and were at first thought to be salmonellae. No Arizona paracolons have been isolated to

date from human or animal sources in this laboratory, but one strain, different from those found in Indian bones, has been isolated from an abattoir in Glamorgan. Other strains have been isolated from an abattoir in East Anglia (Dixon, Personal Communication). It would not, therefore, be surprising if infection in animals due to this group were to be discovered from time to time.

Summary

A survey of crushed bone of Indian origin for salmonellae and Arizona paracolons is described.

Fifty-six out of 57 samples yielded salmonellae; 56 salmonella serotypes, including 5 previously undescribed, were isolated and 8 Arizona paracolon strains.

Of the 56 salmonella serotypes recognized, 38 are known to have been associated with food poisoning in this country.

VI AN OUTBREAK OF FOOD POISONING CAUSED BY SALMONELLA TYPHI-MURIUM, PHAGE-TYPE 12, PROBABLY SPREAD BY INFECTED MEAT

Not all outbreaks of salmonella food poisoning are explosive; some take the form of sporadic cases over a prolonged period of time. On inquiry, no common food and no single food premises are found to be implicated (Harvey and Phillips 1961). Before the development of phage-typing of Salmonella typhi-murium (Felix and Callow 1943; Callow 1959; Anderson 1960), many of the infections comprising such a group of related cases would have been regarded as sporadic. The use of phage-typing now enables the infections to be linked and allows them to be studied as a single episode instead of a series of apparently unrelated incidents of unknown origin (Anderson, Galbraith and Taylor 1961). An outbreak caused by Salmonella typhi-murium, phage type 12, which occurred in South Wales in 1960, illustrates such a pattern of infection.

Investigation of the Outbreak

The human infections occurred over the period May to October 1960 (Table XXV). The geographical distribution of the incidents is shown in Figure II. It will be noted that the main impact was experienced in Cardiff.

The patients were visited by staff of the City Health Department, and inquiries were made as to possible sources of infection. No single item of food could be implicated, but as meat was a possible cause of the outbreak, gauze swabs were placed in open drains in the abattoirs of of Barry and Cardiff (Figure II). These drains were not subject to human pollution and it was considered that

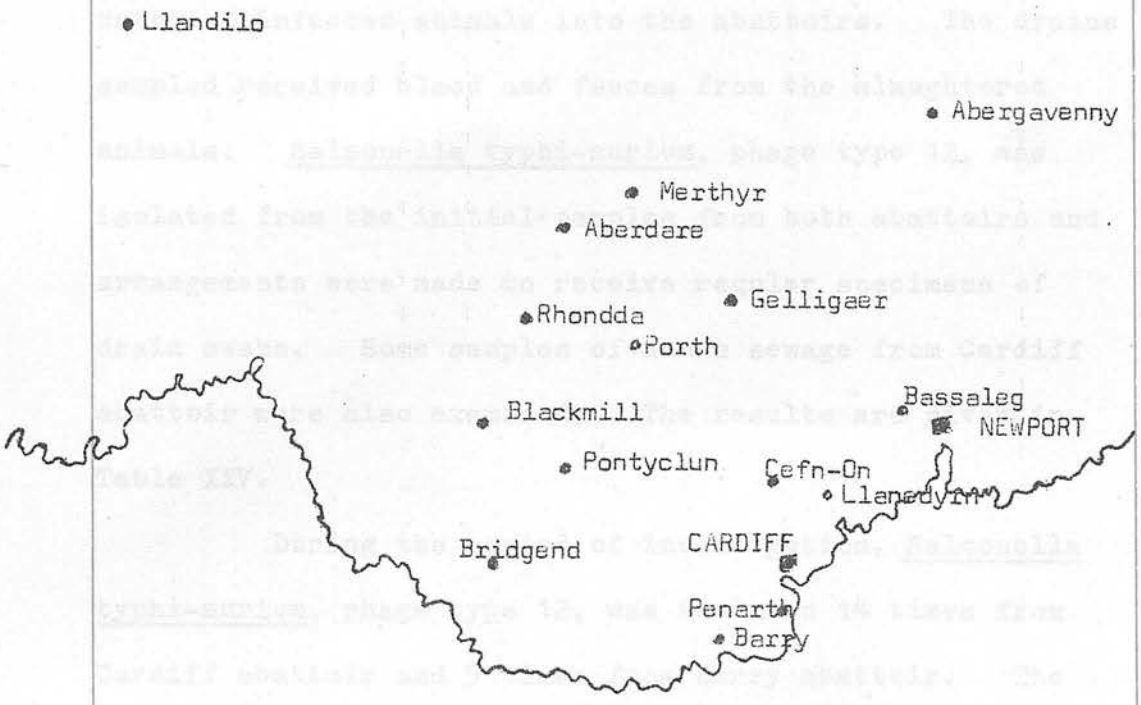
TABLE XXV

Time Relations in the Outbreak - 1960

MONTH	W/E	MAY			JUNE			JULY				AUGUST				SEPTEMBER				OCTOBER				NOVEMBER			
		13	20	27	3	10	17	24	1	8	15	22	29	5	12	19	26	2	9	16	23	30	7	14	21	28	4	11	18	25
Human Incidents - Glamorgan		1	0	0	4	2	1	1	2	1	6	2	1	6	3	2	2	3	2	0	0	1	0	0	1	2	0	0	0	0	0	0
Cardiff Abattoir - Drains Receiving Material from Slaughter									+	+	+	+	+	+	+	+	+	+	+	-	-	+	-	+	-	+	-	-		+
Cardiff Abattoir - Human Sewage											+	+	+	+	+	-	+	-	-			-	-	-	-	-	-	-		-
Cardiff Abattoir * Faeces of Staff - 63 Persons											+ 1																			
Cardiff Abattoir * Pig Faeces - 69 Pigs											+ 1																			
Cardiff Abattoir * Rats - Only One Rat Caught											+ 1																			
Retail Shop Drains - Cardiff										+	+	+							+											
Retail Shops - Cardiff * Food Handlers - 112 Persons											+ 1																			
Barry Abattoir - Drains Receiving Material from Slaughter										+	-	+	+	+	-	+	-	-	-	-	-	-	-	-	-		-	-	-	-

* Figure underneath + sign denotes number of animals or persons found positive

FIGURE II



Geographical distribution of human and animal incidents caused by *S. typhi-murium*, phage-type 12, in South Wales.

Human incidents		Animal incidents on farms		
Place	No. of incidents	Place	Date	Animals infected
Aberdare	1	Llandilo	12 October 1959	Pigs
Barry	4	Abergavenny	30 December 1959	Pigs
Blackmill	2	Bassaleg	7 July 1960	Cows
Bridgend	1	Llanedyrn	18 July 1960	Pigs
Cardiff	81			
Cefn-On	1			
Gelligaer	2			
Merthyr	2			
Penarth	6			
Pontyclun	1			
Porth	2			
Rhondda	2			
	105			

Total human infections = 122
Sporadic cases = 95
Family incidents = 10

isolations of salmonellae from them would indicate the entry of infected animals into the abattoirs. The drains sampled received blood and faeces from the slaughtered animals. Salmonella typhi-murium, phage type 12, was isolated from the initial samples from both abattoirs and arrangements were made to receive regular specimens of drain swabs. Some samples of human sewage from Cardiff abattoir were also examined. The results are given in Table XXV.

During the period of investigation, Salmonella typhi-murium, phage type 12, was isolated 14 times from Cardiff abattoir and 5 times from Barry abattoir. The prolonged isolations of phage type 12 from the Cardiff abattoir specimens over the 10-week period 8th July 1960 to 9th September 1960 is striking. In 5 years of abattoir swabbing, this is the only instance we have encountered of the isolation of the same salmonella strain from consecutive swabs for so long a period. The strain was isolated 14 times from drains receiving material from pig slaughter and 13 times from drains receiving material from cattle slaughter. The human sewage from Cardiff abattoir was sampled regularly from the week ending 22nd July 1960 and was positive on 6 occasions for phage type 12. As the view is sometimes put forward that salmonella isolations from an abattoir may have a human source, the sampling of human sewage at Cardiff slaughter house was regularly continued after the end of the outbreak. This sewage was last found positive at the abattoir in the week ending 2nd September 1960. Since then it has not once been positive over a

period of 30 months, although frequent isolations of salmonellae have been made from drains receiving material from slaughtered animals. It is thus improbable that the salmonella strains isolated from the abattoir had a human origin. The technique of examination was that described previously (Harvey and Phillips 1961).

From the records of the Veterinary Investigation Department, Cardiff, it was found that type 12 had been isolated from animals on three farms in South Wales in 1959 - 1960. The isolations had been made twice from pigs and once from a cow. It was arranged during the outbreak to examine individual cow and pig faeces taken at the city abattoir. Altogether 201 cattle faeces and 69 pig faeces were examined. All the cattle faeces were negative, but one pig faeces was found to be positive for Salmonella typhi-murium, phage type 12. The pig from which the positive specimen was obtained was traced to a farm at Llanedyrn - a village between Cardiff and Newport. The geographical position of all animal incidents on farms is given in Figure II.

One rat was caught in the Cardiff abattoir. Salmonella typhi-murium, phage type 12, was isolated from it.

In a previous group of related cases due to Salmonella typhi-murium, 1a var.3 (Harvey and Phillips 1961), it had been possible to isolate the organism from farm animals in South Wales, from abattoir samples and from local human infections. In the present outbreak, we wished to follow the path ofinfection to retail food premises. As most of the human incidents occurred in

Cardiff, it was decided to examine food shops in the city. The sampling technique used was identical with that previously employed in the drains of abattoirs.

The shops examined were selected either because they were directly associated with notified cases, or because they were located in the neighbourhood of groups of cases. They comprised 54 premises of mixed types.

Swabbing was carried out from July to September 1960. Salmonella typhi-murium, phage type 12, was isolated by the drain-swab technique from 13 butchers' shops and 2 bakehouses. The latter premises sold made-up meat products in the form of sausage rolls as well as confectionery. Both bakehouses belonged to the same firm. Although the organism was not isolated by drain sampling from a further butcher's shop, one of the employees at this shop was found to be excreting phage type 12. This man was also employed as an assistant slaughterman at the Cardiff abattoir. It was possible, therefore, to demonstrate the presence of the type on the premises of 16/54 of the selected food establishments. Details of the premises in which the organism was found are given in Table XXVI.

Other strains of salmonellae were also isolated from these food premises, namely Salmonella typhi-murium, phage types 2c, 1, 1a var.3 and untypable; Salmonella thompson, Salmonella give, Salmonella kiambu, Salmonella derby, Salmonella coley-park, Salmonella binza, Salmonella brandenburg. All these isolations were made from premises dealing in raw or processed meat.

TABLE XXVI

Isolation of Salmonella typhi-murium, Phage Type 12, - Food Premises

Food Premises No.	July 14	July 18-19	July 22	July 26-27	July 31	Aug. 17	Aug. 25	Later	Description of Retail Premises
1	-	+		-				-	Butcher
2	+	-		-				-	Butcher
3	+	+		-				-	Butcher
4		+		-	-				Butcher
5	-	+		-				-	Butcher
6	+	+	-					-	Butcher
7	+	+	+					-	Butcher
8	+		+	-				-	Butcher
9	+	-		-				-	Butcher
10	+	+		+				-	Butcher
11	+	+		-				-	Butcher
12	+	-		-				-	Baker
13	+	-		-				-	Butcher
14				-		-	-	+	Butcher
15 *		-		-					Butcher
16	-	+		-				-	Baker

* Drains receiving material from food preparation rooms negative, but butcher found to be excreting Salmonella typhi-murium, phage type 12

Faecal swabs were taken from all the staff of the infected food premises (total 112). Of these employees, only one was positive - the man mentioned previously who was also an apprentice slaughterman at the abattoir (Table XXVI). This man was clear of infection on 10th August 1960.

Summary

An outbreak of food poisoning caused by Salmonella typhi-murium, phage type 12, in which 122 persons were infected, is described.

Isolation of the same organism from the drains of 2 large abattoirs, from pig faeces at 1 abattoir, and from the floor drains of 15 out of 54 butchers' shops and bakehouses examined, together with records showing that this organism had been recently isolated from pigs and cattle in the neighbourhood, suggested that meat was the vehicle of infection.

VII THE CHANGING PATTERN OF SALMONELLA INFECTION IN ABATTOIRS

In the preceding sections, when I have compared abattoir isolations of salmonellae with human isolations in the same geographical area, the period of time over which the observations were made has been comparatively short. The study of abattoirs by means of Moore's swabs can, however, give other information of a valuable nature, if the time for observation is extended over several years. As an illustration of this, the results derived from 801 swabs are given in Table XXVII. The time of study was six years and the isolations are given monthly for each year. The column at the extreme right hand side of the table gives the average percentage of positive specimens obtained each month. The figure at the foot of this column represents the average percentage of positive samples obtained over the six year period of the observations.

Two important facts can be drawn from consideration of this table. Firstly, it is noted that the incidence of positive results is not constant month by month. There is a greater expectation of finding positive swabs in the third and fourth quarters of the year in contrast to the first and second quarters. This could probably be interpreted as indicating that the environment of abattoirs was more heavily contaminated with salmonellae during the summer and autumn months. The potential danger of salmonellae being disseminated to food premises is, therefore, greatest at this season of the year. This finding could have a bearing on the known peak incidence of

TABLE XXVII

Abattoir Swab Results 1957 - 1962
Cardiff and District

	1957		1958		1959		1960		1961		1962		% Total +
	+	Total	+	Total	+	Total	+	Total	+	Total	+	Total	
January	2	5	2	8	1	7	1	1	1	7	3	11	26
February	0	3	1	9	2	10	1	1	1	16	4	16	16
March	1	4	0	7	2	8	1	2	3	17	6	15	25
April	0	4	1	6	1	12	0	1	0	8	2	9	10
May	2	3	3	6	3	8	2	3	0	13	4	17	28
June	1	3	5	11	5	12	1	1	3	14	2	14	31
July	3	7	0	5	13	19	17	23	1	17	3	18	42
August	1	8	0	0	6	7	16	17	0	10	3	19	43
September	5	13	5	8	12	18	8	20	3	14	5	27	38
October	0	15	6	12	11	16	10	16	4	19	17	32	44
November	8	12	2	10	3	14	6	14	4	18	10	21	37
December	4	12	1	9	3	8	2	5	1	9	7	17	30
Total	27	89	26	91	62	139	65	104	21	162	66	216	
% +	30		29		45		63		13		31		33

salmonella infections in humans in the third quarter of the year.

Secondly, the total annual incidence of positive swabs is seen to vary from year to year. The reason for this is unknown, as yet, but from the practical view point, the annual variation in abattoir incidence could have its effect on human infection. This point after all is of maximum interest to the public health bacteriologist. Newell (1959), suggested that drain swabbing of specific industrial plants could be used to provide information on the proportion of salmonella types present in a community and the changes in types over a period of time. A variation on this idea is shown in Table XXVIII. In this

TABLE XXVIII

Abattoir and Human Infections Caused by Certain Serotypes in Glamorgan 1957 - 1962

Serotype or Phage Type		1957	1958	1959	1960	1961	1962
Salmonella agama	Abattoir	0	0	1	0	0	0
	Human	0	0	1	0	0	0
Salmonella brandenburg	Abattoir	0	0	0	6	5	0
	Human	0	0	0	2	1	1
Salmonella derby	Abattoir	0	4	0	1	0	6
	Human	0	3	0	0	1	2
Salmonella heidelberg	Abattoir	0	0	2	0	6	21
	Human	0	2	3	2	23	4
Salmonella typhi-murium Total Isolations							
	Abattoir	0	9	35	52	6	29
	Human	21	98	81	204	63	86
Phage type 1a var.3	Abattoir		2	0	1	0	0
	Human		17	6	11	0	0
Phage type 1 var.5	Abattoir		0	4	5	0	0
	Human		0	4	12	1	2
Phage type 12	Abattoir		0	0	40	2	1
	Human		0	0	121	1	1
Phage type 12a	Abattoir		0	0	0	0	27
	Human		0	0	1	0	11

The abattoir figures refer to isolations and the human to incidents

table a selected number of serotypes are listed and the number of isolations made in the local abattoirs and in the local community compared. The development of phage typing of Salmonella typhi-murium (Felix and Callow 1943; Callow 1959; Anderson 1960), has enabled me to include in Table XXVIII isolations of certain specific types of this organism and to compare them with corresponding types in the local human population. It is evident from this

table, that changes in abattoir isolations roughly reflect changes in human salmonella incidents due to certain salmonella species. Occasionally, although there is no correlation between an abattoir isolation in Cardiff and district and the finding of the same serotype in the local population, yet there is a similarity in pattern to the isolations in humans for England the Wales as a whole.

It is equally important to note that other serotypes commonly isolated from human beings show no correspondence at all to serotypes isolated from the abattoir. Such species are possibly commonly disseminated by vehicles other than local butcher meat. By such studies, on a large scale, it may be possible to learn more of the path and vehicle of salmonella infection. One such study has recently been carried out (Report 1964). The epidemiology of salmonellosis should not be thought of in static terms. It is a dynamic disease in that it changes year-by-year under the influence of various factors. The decline in Salmonella thompson infection with the decline in Chinese frozen egg imports during the war is an illustration of this. The presence or absence of certain serotypes in imported and home produced animal food in any one year may well determine the serotype pattern in farm animals and human beings in the same and subsequent years in the United Kingdom. It is obvious that further work is required to explain this changing picture of salmonella epidemiology. At present it is only possible to make guesses as to its causation.

CONCLUSION

In Part II of this thesis, the sections represent a series of studies carried out over the period 1954 to 1962. The individual sections may be regarded as sequential steps in the development of a single theme. It is perhaps pertinent, at this stage, to indicate briefly the train of thought governing the progressive stages by which the complete study evolved.

At the time when the early observations, which comprise Section I, were being made, it was fairly commonly accepted that a human carrier was to be sought for, whenever a food poisoning episode was being investigated. A shift in emphasis took place owing to the work of Thomson (1953) and strong evidence was brought forward that the part played by temporary human excreters of salmonellae in the initiation of outbreaks of infection was less important than had been supposed. The discovery that salmonellae survived for prolonged periods in sewers led to a change in the interpretation of positive isolations from sewage, as there was no way of knowing whether such isolations were evidence of the existence of a human being still excreting salmonellae. This being so, it seemed rather profitless to attempt to discover <u>human foci</u> of salmonellosis by "haphazard" sewage examination. Investigations, therefore, narrowed their scope to the examination of sewage specimens from bakehouses which had long been recognized as of importance in the spread of salmonella infection. After six months spent in the examination of sewage from bakeries, it was quite obvious

that salmonellae could be isolated with fair regularity from this material. I, therefore, suggested to the Cardiff City authorities that it might be worth while ascertaining in greater detail from which drains these salmonellae were entering the sewage system of the bakery. This suggestion was complied with and separate examination of sullage and sewage drains was instituted. It was immediately obvious that many positive isolations of salmonellae could be made from open drains, unconnected with the human sewers at the sampling point, but ultimately draining lower down into the sewage system. The drains found positive were those receiving spilt liquid egg used in the bakery. It was, therefore, evident that the isolation of salmonellae from a bakery environment could sometimes be traced to an ingredient used in cake manufacture and was not evidence of the existence of a human focus. Further studies showed, however, that occasionally members of the bakery staff did in fact become infected for short periods and, therefore, could not be altogether excluded from consideration in investigating outbreaks of salmonella infection due to bakery products. The investigations started in bakehouses, were later extended to abattoirs. This resulted in the discovery of a link between sporadic cases of salmonella infection and the salmonella serotypes isolated from the abattoir environment.

Simultaneously with this survey, I carried out an investigation on the salmonella serotypes isolated from the River Taff, which drains a considerable area of Glamorgan.

The wide range of serotypes encountered indicated the existence of a considerable salmonella problem in the county and supplemented the knowledge of infection derived from the study of routine samples of human faeces. The investigations of Walker (1957) on fertilizers and animal feeding stuffs led as a natural sequence to the examination of samples of Indian crushed bone as a possible explanation of this wider salmonella problem. This material is used in animal food in the south west of the United Kingdom and the survey, when completed, showed the general correspondence between some of the serotypes isolated from crushed bone, human beings and animals.

The development of phage typing of Salmonella typhi-murium enabled me to correlate some of the later abattoir isolations more effectively with corresponding infections in human beings. It also enabled a detailed study to be made of a series of incidents due to Salmonella typhi-murium, phage type 12, and the tracing of these infections to farm animals, abattoirs and butchers' shops.

Finally, from the six years of survey of abattoirs in South Wales, I was able to estimate the seasonal degree of contamination which might be encountered in a slaughter-house. It seemed from this, that positive results from abattoir drains (unconnected with human sewers), were to be expected more frequently in the summer and autumn months. The potential danger of the abattoir, as a disseminator of infection, would, therefore, appear to be greater in that portion of the year.

Surveys, as detailed above, are of course only a means to an end in that they merely explain the probable path taken by an infective agent from the source to the human host. It is only after completion of such surveys that the vehicle and paths of infection are sufficiently understood for action to be taken. That point has not yet been reached, but a large amount of information has been collected to incriminate meat, eggs and animal food as potential sources of infection. Other vehicles, such as coconut, pet food and organic fertilizers, may also play a part in salmonella epidemiology. The relative importance of each potential source will have to await the discovery of the means to sterilize that source. When effective sterilization of imported and locally produced contaminated materials is possible, we may indeed see a change in the present pattern of human salmonella infection in this country.

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